Scientific Committee on Health Environmental and Emerging Risks

SCHEER

Final Opinion on

The need for non-human primates in biomedical research, production and testing of products and devices (update 2017)

The SCHEER adopted this final Opinion at its plenary meeting on 18 May 2017

ABSTRACT

Following a request from the European Commission, the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) reviewed recent evidence to update the 2009 Opinion of the Scientific Committee on Health and Environmental Risks (SCHEER) on 'The need for non-human primates in biomedical research, production and testing of products and devices'.

This Opinion responds to six main issues in the mandate and highlights the many scientific approaches that could significantly contribute to the replacement, reduction and refinement (3Rs) of Non-Human Primates (NHP) studies and tests. However, there are significant issues that go beyond scientific rationale that prevent widespread adoption and development of alternatives for NHP laboratory use and these are discussed with suggestions of the opportunities to overcome them.

Although the current state of knowledge does not permit to propose a timetable for phasing-out the use of NHP in Europe, the Opinion provides recommendations on how to advance 3Rs for NHP use, such as through alternative methods, training, improvement of techniques and protocols, sharing of knowledge and removal of barriers. Finally, research needs are given.

Keywords:
SCHEER, scientific opinion, non-human primates, biomedical research, toxicity testing, neuroscience, vaccines, infectious diseases, ophthalmology, (xeno)transplantation.

Opinion to be cited as:

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All Declarations of Working Group members and supporting experts are available at the following webpage:

http://ec.europa.eu/health/scientific_committees/experts/declarations/scheer_wg_en

About the Scientific Committees (2016-2021)

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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SCHEER

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1 SUMMARY

In 2009, the SCHER adopted its Opinion on 'The need for non-human primates in biomedical research, production and testing of products and devices'. The SCHER recognised that animals should only be used in medical research when it is unavoidable and validated alternative methods are not available. The SCHER also considered NHPs essential for scientific progress in important areas of disease, biology, research and safety testing. The SCHER requested a regular review of this position that was reflected in Directive 2010/63/EU. Therefore, the European Commission has requested SCHEER to issue in 2017 an update of the 2009 Scientific Opinion.

NHPs are generally considered the best available animal models for addressing particular research questions because of the close phylogenetic relationship with humans. However, research in NHPs represents a serious ethical dilemma which gives rise to a high level of concern from European Union (EU) citizens. Therefore, human interest in potential benefits for mankind must be balanced against avoiding harm to NHPs and adopting ethical limits or boundaries on NHP use. Opinion polls show that the general public are more accepting of animal research where animal use and suffering are minimised, in line with the 3Rs principle (Replacement, Reduction, Refinement) and with the acceptance of replacement of NHPs in experiments as an achievable goal. This would also include clinical trials and the use of *ex vivo* organs. Directive 2010/63/EU requires implementation of the 3Rs during the design and conduct of animal studies. To fully apply the 3Rs and maximise the benefits, there is a need to ensure that as new knowledge, technologies and approaches emerge there is timely assessment and evolution of research strategies, study designs, scientific procedures and husbandry, throughout the lifetime of research projects. Applying the 3Rs has both scientific and economic merit.

If NHPs are considered necessary for certain uses, it is essential to adopt the highest standards of NHP housing and husbandry and to follow best practice in the conduct and refinement of scientific procedures. In addition, experimental design, analysis and reporting are key means of maximising the knowledge gained from animal experiments and avoiding wastage of animals. Appropriate training for all those working with NHPs is essential to ensure compliance with legislation, excellence in science, animal welfare and full implementation of the 3Rs.

It is recognised that tightening of the existing strict EU regulations for NHP use may lead NHP research to transfer to other countries to the detriment of animal welfare. This can be avoided by international cooperation that engages as many stakeholders and organisations as possible to promote the international development of high standards for research and animal welfare and ethical use.

This Opinion responds to six main issues in the mandate and highlights the many scientific approaches that could significantly contribute to the replacement, reduction and refinement of NHP studies and tests. However, there are significant issues that go beyond scientific rationale that prevent widespread adoption and development of alternatives for NHP laboratory use and these are discussed with suggestions of the opportunities to overcome them. Lastly, the SCHEER provides recommendations on how to advance 3Rs for NHP use, specifically on training, improvement of techniques and protocols, sharing of knowledge, removal of barriers and research needs.

1- The areas of research (fundamental, translational and applied) and testing of products and devices in which NHPs continue to be used today

In 2014, 8898 procedures on NHPs were reported in the EU, including first use and reuse of animals. Currently, NHPs are predominantly used in the following areas a) development and safety testing of pharmaceuticals and medical devices, b) treatment and prevention of infectious diseases, c) neuroscience, d) ophthalmology and e) (xeno)transplantation. The first three categories comprise the majority of NHPs used. While NHPs are often the species of choice, their use for development and safety testing of pharmaceuticals and medical devices also meets regulatory requirements.

2- The currently available possibilities by type of research or testing to replace their use either with methods not entailing the use of animals or by using other species of animals including those genetically altered

Progress has been made in identifying opportunities to avoid NHP use where, 1) they are not the relevant species, 2) alternative species are available and 3) other methods can be used. With regard to the study of disease, there is consensus in the scientific community that one model can never fully recapitulate all aspects of human diseases. This implies that a variety of models, animal and non-animal, should be used. If a one-to-one replacement of a test may not be achievable, an integrated testing strategy should should be used in which *in silico*, *in vitro*, *ex vivo* and *in vivo* experiments, and clinical research are used in combination with a weight-of-evidence approach. Recent developments in biomedical research will potentially improve the selection of the most promising candidates for new therapies before further assessment *in vivo*.

Examples of currently available possibilities for replacement are:

• Case-by-case approach for the choice of a second non-rodent species and greater emphasis in regulatory guidelines on the use of alternative methods in the safety assessment of pharmaceuticals.

• Replacement strategies for treatment and prevention of infectious diseases with the development and use of controlled human challenge models for typhoid, *Plasmodium falciparum* malaria and transmission studies with specific influenza strains.

• The potential to use new, high spatial and temporal resolution imaging techniques in humans to replace some cognitive neuroscience experiments performed in NHPs.

• NHPs are no longer considered acceptable organ donors for practical and ethical reasons.

3- The opportunities for the reduction and refinement of NHP use in areas where no replacement can be foreseen in medium- or long-term, as per the principles of the 3 Rs

Researchers are encouraged to increase the yield of data per animal and experimental session and to share data and tissues with other researchers and to publish negative/null results. Greater efforts are needed to assess the degree of pain and distress experienced by NHPs, so that refinements can be implemented effectively. For studies examining the effects of lesions or other interventions within or between groups of animals, factors such as effect size should be considered to reduce sample sizes. Examples of currently available possibilities for reduction and refinement are:

- Safety assessment of chemicals and drugs is being enriched by increasing knowledge and substantial experience of non-animal techniques, which potentially greatly

reduces the number of NHPs used. Additionally, there are experimental clinical pain protocols developed for providing early clinical proof of concept within the boundaries of a phase I clinical trial design (analgesic drugs).

- Increasing costs of drug development of investigational medicinal products favour limited human exposure in exploratory clinical trials, including microdosing.
- Patient-derived induced pluripotent stem cells (iPSCs) have the potential to reduce the number of animal-based preclinical tests and even replace some of them.
- Application of novel sophisticated imaging techniques to study the development of infectious diseases may result in more information from fewer animals.
- Technological developments in neuroscience have enabled refinement of surgical and other procedures e.g., refining and de-sizing the devices used in invasive experiments, improving the anaesthetics and analgesics with faster recovery used in imaging experiments and surgery, non-invasive imaging methods help reduce and refine invasive techniques such as surgery, refinement of food and fluid control protocols and wireless technology have a positive impact on NHP welfare.

4- Identification of specific research areas where effort should be made to advance replacement, reduction and refinement of the use of non-human primates in scientific procedures

There is an urgent need to conduct systematic reviews and meta-analysis of all areas of NHP use. This would potentially significantly reduce the number of NHPs used and the resources required by identifying where they are unsuitable models or where they have contributed very little to current knowledge. Additionally, it may provide evidence for more targeted use of NHPs, which is important for ethics committees[1] and funders of research. Emphasis should also be given to ensuring proper reporting of NHP studies and effective knowledge transfer, focusing NHP research in centres of excellence and the development of suitable, harmonised training courses. Continued work is necessary to develop improved means of assessing pain, suffering and distress in NHPs, including the psychological impact of their use in research. Scientific knowledge about the welfare impact of husbandry and procedures, even after refinement measures have been applied, needs to be factored into harm-benefit assessments.

Examples of efforts needed in specific research areas are:

- To progress towards complete replacement of NHPs in drug safety testing, it will be necessary to gain new insights into molecular biology, including a better understanding of signalling pathways, modelling and bioinformatics and further research into integrated testing strategies, including the identification of Adverse Outcome Pathways (AOPs) leading to human diseases. Additional work is needed on new models for investigating abuse potential, assessment of reproductive toxicity and characterisation of the safety of biopharmaceuticals.
- Further research is necessary into 1) the relevance and limitations of the use of NHP models for infectious diseases and therapies, 2) the optimisation of novel non-NHP animal models, such as humanised mice and 3) development of new techniques such as organoïds and –omics technology.
- In neuroscience, progress is required in refinement of awake, behaving

[1] The role of the ethics committees is to do comprehensive NHP project evaluations which address all aspects of NHP programs including ethical considerations

electrophysiology studies. There should also be a focus on significantly improving the spatial and temporal resolution of non-invasive imaging technologies to enable the replacement of electrophysiological studies in NHPs.

5- The scientific viewpoint on when their use would no longer be necessary, considering the type of research and areas of testing with a view to the establishment of a specific phasing-out time-table where possible

A number of factors determine the timetable for complete replacement of NHP use, among others:

- Availability of funding and resources for developing alternatives to NHP models and ensuring they are fit for purpose.
- Progress in the formal validation of alternative test methods within the regulatory arena and in reducing the timescale and bureaucracy associated with this process;
- Lack of regulatory and guideline harmonisation between countries and the condition that is often included that an alternative method must be formally validated and accepted by regulatory authorities before it can be used.
- New demands for NHP use in science, such as emergence and re-emergence of infectious diseases where NHPs are the only relevant model.
- While NHPs have been advocated by WHO as better models for Biosimilars because they have a higher tolerance for human proteins, EMA and FDA in April 2015 adopted guidelines proposing a tiered approach suggesting that only *in vitro* studies are acceptable.
- The risk aversive nature of society makes it difficult to move away from familiar methods to new alternative methods where there is less historical data to fall back on.

This wide spectrum of positive and negative incentives for NHP use makes it difficult to predict a timetable for complete replacement for each of the research areas.

6- Potential implications for biomedical research (e.g., immune based diseases, neurodegenerative disorders, infectious diseases and serious diseases) should the use of non-human primates be banned in the EU.

Recognising the high levels of public concern about NHP research, regulatory authorities in some world regions have also adopted ethical limits or boundaries on NHP use. However, the close phylogenetic relationship of NHPs with humans makes them the best available animal models for addressing particular research questions.

Therefore, there is consensus within certain sections of the scientific community that, where alternatives do not exist, appropriate use of NHPs remains essential in some areas of biomedical and biological research and for the safety assessment of pharmaceuticals. As long as sufficiently validated alternatives are not available, a total ban would make further progress in such research and some safety studies impossible, at least in Europe.

Since animal welfare standards for laboratory NHPs are on average higher in many European countries than in other parts of the world, it follows that if NHP research is forced outside of Europe then there would likely be a net decrease in animal welfare. This could also have an impact on the quality of the research, on public health and accessibility of treatments developed under different standards and on local economy.

As long as a total ban is not feasible, when communicating about NHP use with the

public, the scientific community should provide an accurate description of the benefits, harms to animals and limitations of such research, and be realistic about the potential outputs and impacts.

2 MANDATE

2.1 BACKGROUND

Directive 2010/63/EU on the protection of animals used for scientific purposes[2], revising and replacing Directive 86/609/EEC, was adopted in 2010. It provides for controls of the use of live animals for scientific purposes including a systematic project evaluation and authorisation, sets binding standards for housing and care as well as for the education, training and competence of personnel both handling animals and supervising the experiments.

Due to their genetic proximity to humans and highly developed social skills, the use of non-human primates in scientific procedures raises specific ethical questions and practical problems in terms of meeting their behavioural, environmental and social needs in a laboratory environment. Furthermore, the use of non-human primates for scientific purposes is of the highest concern to the citizens. As a result, the use of non-human primates attracted significant attention during the review of the Directive.

In this context, it is useful to recall the 2002 Scientific Steering Committee, SSC, report[3] highlighting the continuing need to use non-human primates in biomedical research. This was followed by the European Parliament declaration adopted in 2007 urging the Commission to

1. Make an end to the use of great apes and wild-caught monkeys in scientific experiments;
2. Establish a timetable for replacing with alternatives the use of all primates in scientific experiments.

The Commission stated in its response[4] to the European Parliament (EP) that with the current scientific knowledge at the time a timetable with a fixed deadline to phase out all use of non-human primates in the area of biomedical research was not possible. However, the Commission also recognised that science is evolving rapidly in this field and novel technologies, such as genomics and computer modelling, were gradually emerging.

As a result of these events, DG ENV requested the Scientific Committee on Health and Environmental Risks (SCHER) to issue an opinion on the status of alternatives for the use of non-human primates to facilitate an informed debate during the negotiations of the European Parliament and the Council on the revision of the Directive.

SCHER adopted its opinion in January 2009[5]. The SCHER opinion concurred with the Commission's view seeing no scientific reasons to support a discontinuation of the use of non-human primates in basic and applied research, or in the development and testing of

[2]OJ L 276, 20.10.2010, p. 33–79
[3]The Scientific Steering Committee: "The need for non-human primates in biomedical research", statement adopted 4-5 April 2002: http://europa.eu.int/comm/food/fs/sc/ssc/out253_en.pdf
[4]http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm
[5]http://ec.europa.eu/environment/chemicals/lab_animals/pdf/scher_o_110.pdf

new drugs. The opinion assessed the different areas in which non-human primates were being used. It examined areas for which partial or full replacement could be foreseen, and discussed the opportunities for the reduction and refinement of the use of non-human primates in areas where no replacement could be expected in medium or long term.

Use of non-human primates in scientific procedures today

There has been a decrease in the use of NHPs compared to the situation in 2008. According to the latest available statistics of the EU from 2011, around 11 million animals were used in scientific procedures in the EU. Of these, approximately 6,000 were NHPs, compared to almost 10,000 in 2008.

From a scientific point of view, the use of NHPs, at the time of the SCHER report, was considered essential for scientific progress in a number of important areas of disease biology, research and in safety testing, including:

– Development of pharmaceuticals, in particular safety testing, to assess potential toxicity in animals to identify unacceptable adverse reactions in humans;

– Understanding the pathophysiology of infectious diseases such as HIV/AIDS, where the NHP was considered the only susceptible species and therefore the only useful animal model to study the disease, and to develop safe and effective vaccines and therapies;

– Learning how complex brains of primates, humans included, are structured and function. Again, NHPs were considered the best model due to their close similarity to humans with regard to brain complexity and function;

– Developing and testing xenotransplantation methodologies.

This is reflected in the EU statistics which show that 56% of the non-human primates were used for the purposes of toxicological and other safety evaluations, 10% in the area of basic research and 23% in applied research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine.

Need for an update

A number of recommendations put forward by SCHER are reflected in the Directive. The Directive restricts the purposes for which non-human primates can be used, requires scientific justification that no other species can be used and requires more stringent inspections of establishment keeping or using non-human primates. An individual history file must be kept for each non-human primate and projects using non-human primates cannot to be authorised using simplified administrative procedures.

In addition, any work involving the use of non-human primates is subject to a retrospective assessment at the end of the project. The use of wild-caught non-human primates for scientific purposes is prohibited and specific measures are required to move towards using only second (or higher) generation purpose-bred non-human primates.

Finally, SCHER recommended that their position should be regularly reviewed in the light of alternative approaches that are constantly being developed.

This is reflected in Recital 49 of the Directive that provides that "*Technical and scientific advancements in biomedical research can be rapid, as can the increase in knowledge of factors influencing animal welfare. It is therefore necessary to provide for a review of*

this Directive. Such review should examine the possible replacement of the use of animals, and in particular non-human primates, as a matter of priority where it is possible, taking into account the advancement of science…."

The legal obligation for the review is embedded in the first paragraph of Article 58:

"The Commission shall review this Directive by 10 November 2017, taking into account advancements in the development of alternative methods not entailing the use of animals, in particular of non-human primates, and shall propose amendments, where appropriate."

2.2 TERMS OF REFERENCE

In view of the above, the Commission asks the SCHEER to issue a scientific opinion, updating the SCHER opinion of 13 January 2009, "The need for non-human primates in biomedical research, production and testing of products and devices", on:

- The areas of research (fundamental, translational and applied) and testing of products and devices in which non-human primates continue to be used today;
- The currently available possibilities by type of research or testing to replace their use either with methods not entailing the use of animals or by using other species of animals including those genetically altered;
- The scientific viewpoint on when their use would no longer be necessary, considering the type of research and areas of testing with a view to the establishment of a specific phasing-out time-table where possible;
- The opportunities for the reduction and refinement of their use in areas where no replacement can be foreseen in medium or long term as per the principles of the Three Rs;
- Identification of specific research areas where effort should be made to advance replacement, reduction and refinement of the use of non-human primates in scientific procedures.
- Potential implications for biomedical research (e.g., immune based diseases, neurodegenerative disorders, infectious diseases and serious diseases) should the use of non-human primates be banned in the EU.

SCHEER's opinion would be appreciated by the end of December 2016 to contribute to the preparation of Commission Review of the Directive as provided by Article 58 of the Directive with a deadline of 10 November 2017.

3 OPINION

3.1 Overview

In 2009, SCHER adopted its first Opinion on 'The need for NHPs in biomedical research, production and testing of products and devices'. The SCHER acknowledges that animals should only be used in medical research when it is unavoidable and validated alternative methods are not available. The SCHER also considered NHPs essential for scientific progress in important areas of disease, biology, research and safety testing. The SC requested a regular review of this position that was reflected in Directive 2010/63/EU. Therefore, the European Commission requested SCHEER to issue an update of the 13 January 2009 Scientific Opinion. This current Opinion addresses six areas from the Commission's mandate on "the need for non-human primates in biomedical research, production and testing of products and devices" (Section 3.2).

NHPs are generally considered the best available animal models for addressing particular research questions because of the close phylogenetic relationship with humans. However, this similarity renders the acceptance of inducing pain or distress as a consequence of their use in scientific procedures even more debatable/challenging than for other species of animals. Hence, research in NHPs represents a serious ethical dilemma which gives rise to a high level of concern from EU citizens. Therefore, human interest in potential benefits for mankind arising from NHP research must be balanced against avoiding harm to NHPs and adopting ethical limits or boundaries on NHP use.

Opinion polls show that the general public is more accepting of animal research where animal use and suffering are minimised, in line with the 3Rs principle. If NHPs are considered necessary for certain uses, it is essential to adopt the highest standards of NHP housing and husbandry[6] and to follow best practice in the conduct and refinement of scientific procedures. In addition, experimental design, analysis and reporting are key means of maximising the knowledge gained from animal experiments and unnecessary use. Appropriate training for all those working with NHPs is essential to ensure compliance with legislation, excellence in science, animal welfare and full implementation of the 3Rs.

It is recognised that tightening of the existing strict EU regulations for NHP use may lead NHP research to transfer to other countries to the detriment of animal welfare. Decreased animal welfare may also lead to less reliable results. This can be avoided by international cooperation that engages as many stakeholders and organisations as possible to promote the international development of high standards for research and animal welfare and ethical use.

In this section, SCHEER will address each of the six mandate issues beginning with a general section that lists topics relevant for all research areas, followed by research area-specific topics. The scientific rationale, including supporting references, for this new opinion is given in Section 6. Recommendations are given in Section 7.

[6]Upgraded legal obligations concerning space allowances for NHPs were taken effect 1.1.2017 Dir 2010/63/EU, Annex III

3.2 The areas of research (fundamental, translational and applied) and testing of products and devices in which non-human primates continue to be used today

Currently, NHPs are predominantly used in the following areas a) development and safety testing of pharmaceuticals and medical devices, b) treatment and prevention of infectious diseases, c) neuroscience, d) ophthalmology and e) (xeno)transplantation. The first three categories comprise the majority of NHPs used. While NHPs are often the species of choice, their use for development and safety testing of pharmaceuticals and medical devices also meets regulatory requirements.

3.3 The currently available possibilities by type of research or testing to replace their use either with methods not entailing the use of animals or by using other species of animals including those genetically altered

General issues

Significant progress has been made in identifying opportunities to avoid NHP use where, 1) they are not the relevant species, 2) alternative species are available and 3) other methods can be used. Where NHP use is scientifically justified, progress has been made in identifying models in which the human target molecule is present in NHPs but not in other animals and in improving study designs to use fewer animals. Greater progress would be made in the replacement of NHP experiments, if this was accepted as an achievable goal by the scientific community. Importantly, decisions about the need for NHP use should be made case-by-case based on harm-benefit assessment taking into account ethics and scientific rationale.

With regard to the study of disease, there is consensus in the scientific community that a single model can never fully recapitulate all aspects of human diseases. The type of scientific question asked and the methodology used will determine how useful and how predictive the results obtained are. This implies that a variety of models, animal and non-animal, should be used to address different aspects of the same disease. If one-to-one replacement of a test may not be achievable, an integrated testing strategy in which *in silico*, *in vitro*, *ex vivo* and *in vivo* experiments and clinical research are combined in a weight-of-evidence approach, could fulfil the task. Moreover, recent developments in biomedical research, e.g., cell culture techniques, *in silico* modelling, –omics, organoids and novel fully artificial whole-body models will potentially improve the selection of the most promising candidates for new therapies before further assessment *in vivo*.

Notably, substitution of NHPs with rodents or other laboratory animal species is not replacement as defined by Russell and Burch (1959). However, this may be ethically desirable if the available evidence indicates that the non-primate species is likely to suffer less harm.

Development and safety testing of pharmaceuticals and medical devices

Replacing NHP models in new medicine development and safety testing has the potential to significantly reduce the number of NHPs used in European laboratories, as regulatory use and routine production are consistently the areas of greatest NHP use. Examples are *in vitro* and *in silico* models for studying liver injury and testing for idiosyncratic adverse drug reactions, a new mouse model for understanding human-specific menstrual and pregnancy associated disease, and swine models as second species next to rodents.

Although international regulations specify that non-rodent species should be used for safety assessment of pharmaceuticals prior to their being used for the first time in man and that one of these species can be an NHP species, a significant change in emphasis is that the need to use NHPs should now be considered on a case-by-case basis. ICH guideline S6 (R1) on preclinical safety evaluation of biotechnology-derived pharmaceuticals, in force since December 2011, emphasises the use of *in vitro* alternative methods for safety evaluation and opening the way to the replacement of current standard methods. NHPs may be substituted by transgenic animals (usually mice) or by the use of homologous proteins in rodents. The EU Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues, which came into effect on 1 December 2012, states "the conduct of toxicological studies in NHPs is usually not recommended".

Arguments against phasing out animals in safety testing of pharmaceuticals are: incomplete knowledge in new models of integrated body systems and pathophysiology, poor representation of pharmacokinetics by isolated *in vitro* systems (so far only partially overcome by using PB-PK modelling) and difficulties in extrapolating from *in vitro* data to benchmark doses, which is vital for human risk assessment. Human models are progressing in translational medicine; however, they are not yet a substitute for well-designed animal models. Important progress is still required to develop and validate new alternative methods and to validate them, before obtaining scientific acceptance and regulatory recognition.

Treatment and prevention of infectious diseases

Infectious disease models in NHPs, because of the similarity of NHP and human immune systems, resemble human disease more closely than in other animal models. However, NHP infectious disease models are used when the course of disease resembles human disease with a similar host range of cells, organs or tissues involved and reflect the host response in humans. Progress has been made in replacement strategies for treatment and prevention of infectious diseases with the development and use of controlled human challenge models for typhoid, *Plasmodium falciparum* malaria and transmission studies with specific influenza strains. Other examples in development, which might offer new possibilities for vaccine research, include a human attenuated Tuberculosis (TB) challenge model. These studies are particularly important because of the many ethical and safety barriers that prevent human challenge studies with virulent pathogens. It is not always possible to study fundamental aspects of the disease and to perform in depth analysis of host-pathogen interactions in human volunteers or patients. In some cases, non-NHP animal models, for example, new genetically modified mouse models may substitute for NHPs. Importantly, it is unlikely that new technologies will negate the need for infectious NHP models in the near future due to emerging and re-emerging pathogens.

Neuroscience

In behavioural neuroscience studies, many of the procedures used to monitor and manipulate brain elements are invasive and therefore, only rarely is it considered ethical to carry out these procedures in healthy humans. Opportunities for minimal risk microelectrode recording should be utilised with the patient's informed consent and ethics committee approval. However, data from an injured human brain should be carefully interpreted and cannot replace *in vivo* controlled studies in animals.

There is a potential to use imaging studies in humans to replace some cognitive neuroscience experiments currently being done on NHPs. However, fMRI measures can only indirectly reflect neuronal activity and imaging techniques require further development to completely replace studies in NHPs and other animals.

In vitro disease modelling possibilities have expanded and have potential for replacement, e.g., microfluidic chambers/brain chips, mixed cell cultures, patient-derived-induced pluripotent stem cells (iPS cells), "mini-brains" or organoids. The absence of a blood-brain-barrier, a vascular system and an immune system, however, are major limitations for complex pathophysiological investigations.

Other uses: Ophthalmology

NHPs represent a more relevant model for the discovery and development of new therapeutic strategies as compared with other species. No currently available rodent, *in vitro* or *in silico* model appears to recapitulate the architectural features of the primate eye (i.e., the macula) or the complex structural and functional interactions of retinal cells. New, more pertinent models of ocular pathology are currently being developed in NHPs and new gene therapy strategies are being tested in NHPs before their translation to the clinic. However, good progress is being made developing suitable *in vitro* models of retinal defects for screening and efficacy testing of new therapies. The potential for replacement is encouraging, especially through the use of human stem cells in developing human cell-based models of visual pathways, particularly the retina, and models of macular degeneration (Singh *et al.,* 2012).

Other uses: (Xeno)transplantation

NHPs are no longer considered acceptable organ donors for practical and ethical reasons. Pigs have the potential to be the prime candidates for organ donation. However, due to their anatomic and immunologic similarity to humans, NHPs have been used as recipients to investigate the fundamental aspects of organ transplantation and may be required in the future to evaluate new immunosuppressive therapies and new methods to prevent organ rejection. The scientific justification of the use of NHPs as recipients should still be examined on a case-by-case basis and should take into account the *in silico*, *in vitro* and *in vivo* data acquired in a non-primate species.

3.4 The opportunities for the *reduction and refinement* of their use in areas where no replacement can be foreseen in medium or long term as per the principles of the Three Rs

General issues

Researchers are encouraged to increase the yield of data per animal and experimental session, to share data and tissues with other researchers and to publish negative/null results. Greater efforts are needed to assess the degree of pain and distress experienced by NHPs, so that refinements can be implemented effectively. For studies examining the effects of lesions or other interventions within or between groups of animals, factors such as effect size should be considered to reduce sample sizes.

Development and safety testing of pharmaceuticals and medical devices

Safety assessment of drugs is being enriched by increasing knowledge and substantial experience of non-animal techniques, which greatly reduces the number of NHPs used. Additionally, there are experimental clinical pain protocols developed for providing early

clinical proof of concept, within the boundaries of a phase I clinical trial design (analgesic drugs).

Exploratory Clinical Trials, including microdosing, were established by regulatory authorities to reduce the nonclinical testing prior to First in Human (FIH) clinical trials (ICH M3 (R2)). These studies allow an early decision based on clinical data with associated time and cost savings, and reduced drug manufacturing requirements (Burt *et al.,* 2016).

New applications of human microdosing studies to areas beyond exploratory pharmacokinetic data could significantly reduce the number of unsuccessful candidate drugs from progressing into the full developmental process and as a consequence reduce the number of pre-clinical animal tests. This includes using microdosing to investigate drug-drug interactions, site of action, pharmacokinetics in vulnerable populations, intravenous data and metabolic profiling. The implementation of human microdosing studies prior to full Phase I clinical trials can eliminate unsuccessful candidates from progressing through the full developmental pathway. As microdosing studies are allowed based on a reduced safety package compared to a full Phase I trial, then both the cost and the number of required animal-based (including NHPs) preclinical test are reduced.

Patient-derived induced pluripotent stem cells (iPSCs) have the potential to reduce the number of animal-based preclinical tests and even replace some of them. They can be used to assess the toxicity and clinical efficacy of drug candidates before they are progressed into market.

Cross-company and cross-sector data sharing has identified efficient study designs for regulatory toxicology studies, helping to reduce NHP use per study/test compound (e.g., fewer dose groups, animals per group, and recovery animals.

Treatment and prevention of infectious diseases

New imaging techniques may enable the study of the development of certain infections, including early effects of infection, and/or therapy over time in the same individual. The application of novel sophisticated imaging techniques may result in more information from fewer animals.

Identifying correlates of protection to avoid the need for progression to severe clinical signs would help to refine infectious disease studies.

Neuroscience

Technological developments have enabled refinement of surgical and other procedures within the neurosciences, *e.g.*:

- Refining and de-sizing the devices used in invasive experiments,
- Using wireless techniques to record neuronal activity,
- Using positive training techniques to reduce stress in housing management and procedures.
- Improving the anaesthetics and analgesics with faster recovery used in imaging experiments and surgery,
- Using non-invasive imaging methods that help reduce and refine invasive techniques such as surgery,
- Refining food and fluid control protocols to minimise the impact on NHP welfare.

3.5 Identification of specific research areas where effort should be made to advance replacement, reduction and refinement of the use of non-human primates in scientific procedures

General issues

There is an urgent need to conduct systematic reviews and meta-analysis of all areas of NHP use and to build up public databases so that no duplication is allowed. This could potentially significantly reduce the number of NHPs used and the resources required by identifying where they are unsuitable models or where they have contributed very little to current knowledge. Additionally, research funders should ensure that NHP proposals are only funded where there is no suitable alternative approach, and where there is a high likelihood of scientific, medical or social benefit. Emphasis should also be given to ensuring proper reporting of NHP studies and effective knowledge transfer, to maximise the value of funded research.

Importantly, it is advisable to ensure the harmonisation and implementation of regulations leading to improvements of replacement, reduction and refinement. It is essential to stimulate changes in both scientific and societal attitudes and in scientific practice by improving and expanding the 4Cs (Commitment, Communication, Cooperation and Coordination). This may require improvements to peer review processes for project proposals, (e.g., appointment of panel members with expertise in alternatives to NHPs, statisticians and clinicians; amendments to application forms, guidance and training, to ensure provision of quality information about the justification for NHP use, animal numbers and experimental design, with robust scrutiny of this information).

It is recommended that consideration be given to focusing NHP research in centres of excellence, defined by performance of excellent research in combination with the provision of optimal care and use of NHP, and improving existing networks for information sharing. Moreover, researchers and animal care staff must ensure that they keep abreast of the latest techniques that enable reduction in animal numbers and of the refinement of existing methods and techniques to reduce suffering, and put this evidence base into practice. Consideration should be given to continuous development of an accredited training course for those working with NHPs, harmonised across Member States, and improved access to training in experimental design. Work needs to continue to develop improved means of assessing pain, suffering and distress in NHPs, including the psychological impact of their use in research. Scientific knowledge about the welfare impact of husbandry and procedures, even after refinement measures have been applied, needs to be assessed and factored into harm-benefit assessments. A high quality social and physical environment for NHPs must always be assured, beyond the scope of a particular study.

Development and safety testing of pharmaceuticals and medical devices

Progress has been made in identifying opportunities to avoid NHP use where they are not a relevant species or alternative species can be used and in identifying efficient study designs using fewer animals where NHP use is scientifically justified. In addition, progress has been made in identifying situations where nonclinical testing can be conducted in a single relevant species. To progress towards complete replacement of NHPs in safety testing, it will be necessary to gain further insight into molecular biology, including a better understanding of signalling pathways, modelling and bioinformatics and additional research into integrated testing strategies**.**

More work is needed on new models for investigating abuse potential, the assessment of reproductive toxicity and the characterisation of the safety of biopharmaceuticals.

Treatment and prevention of infectious diseases

Research is necessary for

- Determining the relevance and limitations of the use of NHP models for infectious diseases and therapies,
- The improvement of techniques and NHP characterisation to further refine models and reduce the number of NHPs required,
- The optimisation of novel non-NHP animal models, such as humanised mice, and
- Development of new techniques such as organoids and –omics technology to further reduce the current need to use NHPs to study important infections.

Neuroscience

There has been progress with refinement of awake, behaving electrophysiology studies, but further progress is required, given the high impact on animal welfare. The basic paradigm for study of brain activity at the single cell level involves repeated electrode penetration, prolonged restraint, fluid or food control, and surgical procedures to implant head fixation and recording or other devices. There needs to be a focus on significantly improving the spatial and temporal resolution of non-invasive imaging technologies in order to refine and ultimately replace this use of NHPs.

Other uses: Ophthalmology

For advanced replacement techniques in vision research and to learn more about the structure and function of the visual cortex, new technology is necessary for whole organ eye-culturing, *in vitro* and *in silico* models and simulations.

3.6 The scientific viewpoint on when their use would no longer be necessary, considering the type of research and areas of testing with a view to the establishment of a specific phasing-out time-table where possible

General Issues

A number of factors determine the timetable for complete replacement of NHP use:

- The availability of funding and resources for developing alternatives to NHP models and ensuring they are fit for purpose. Criteria for prioritisation of areas of further research could be used, e.g., the absolute number of the NHP used and the severity of procedures.
- The progress in the formal validation of alternative test methods within the regulatory arena and in reducing the timescale and bureaucracy associated with this process.
- Lack of regulatory harmonisation both within and across sectors and the condition that is often included that an alternative method must be formally validated and accepted by the scientific community and regulatory authorities before it can be used
- The progress to validate new non-animal models against existing animal models.
- A continued prominent role and investment of the EU in promoting and implementing alternative methods.
- The extent to which justification for the use of alternative models is laid down in internationally accepted guidelines.

- The risk aversive nature of society makes it difficult to move away from familiar methods to new alternative methods where there is less historical data to fall back on.
- Factors related to scientific practice and career progression where dynamics such as competition, the reputation and track record of researchers (in terms of grants and publications), and entrenchment discourage switching from NHPs to alternative (animal and non-animal) models.
- New demands for NHP use in science, such as emergence and re-emergence of infectious diseases where NHPs are the only relevant model, and biosimilars for which NHPs have been advocated by WHO as better models because they have a higher tolerance for human proteins. It is, however, noted that EMA and FDA in April 2015 adopted guidelines proposing a tiered approach and suggesting that only *in vitro* studies are acceptable (Chapman *et al.,* 2016).

This wide spectrum of positive and negative incentives for NHP use makes it difficult to predict a timetable for complete replacement for each of the research areas.

3.7 Potential implications for biomedical research (e.g., immune based diseases, neurodegenerative disorders, infectious diseases and serious diseases) should the use of non-human primates be banned in the EU

Recognising the high levels of public concern about NHP research, regulatory authorities in some world regions have also adopted ethical limits or boundaries on NHP use. However, the close phylogenetic relationship of NHPs with humans makes them the best available animal models for addressing particular research questions.

Therefore, there is consensus within certain sections of the scientific community that, where alternatives do not exist, appropriate use of NHPs remains essential in some areas of biomedical and biological research and for the safety assessment of pharmaceuticals. For example: NHPs can play a key role in drug development due to their similarity to humans with regard to sensory organs, hormonal systems, reproduction, immune system etc., to evaluate efficacy and safety, especially for biopharmaceutical compounds. There has been progress in a move toward a non-animal predictive mechanism-based approach, e.g., for testing of drugs for reproductive toxicity, but there are still obstacles to overcome in terms of regulatory acceptance and scientific validity.

In spite of this, NHPs should only be used when it is scientifically demonstrated that none of the other non-rodent species commonly used in safety testing is appropriate for the purpose of the study.

As long as sufficiently validated alternatives are not available, a total ban would make further progress in such research and some safety studies impossible, at least in Europe. On the other hand, a ban may lead to acceleration of investment into the development of alternative methods as seen for cosmetics (European Commission, 2016).

However, a ban in Europe would likely force NHP research outside of Europe and since animal welfare standards for laboratory NHPs are on average higher in many European countries than in other parts of the world, it follows that there would likely be a net decrease in animal welfare. Though some companies have developed global animal welfare policies, there are concerns that European scientists are transferring their research programmes outside the EU to countries where welfare/scientific standards

would not be judged ethically acceptable in Europe. This could have an impact also on the quality of the research, on public health and accessibility of treatments developed under different standards and on local economy

As long as a total ban is not feasible, when communicating about NHP use with the public, the scientific community should provide an accurate description of the benefits, harms to animals and limitations of such research, and be realistic about the potential outputs and impacts.

4 MINORITY OPINION

None.

5 DATA AND METHODOLOGY

An initial literature research was undertaken by the SCHEER in July 2016 to identify key publications for this Opinion. The aim of the literature review was to collect publications from January 2009 to July 2016 for this Opinion. The following terms were used for the literature review in the title, abstract, key word fields: "non-human primates" AND (("human+health" AND "relevance") OR (ethics OR "alternative+methods" OR "research+needs")) resulting in 26 hits for publications. The following documents were included: peer-reviewed articles, journal entries, reviews, and public polls. The SCHEER WG included additional relevant publications in areas of their expertise.

A call for information[7] was published on 1 January 2009 for collecting papers on new technologies used to replace, reduce and refine the use of non-human primates in biomedical research, production and testing of products and devices. The Call for information was opened between 8 June and 3 July 2016. There were 19 submissions containing more than 100 papers from individual scientists, research organisations, professional societies, pharmaceutical industry and animal protection organisations. Submitted information was considered for the Opinion, if it was relevant for the scope of the Opinion.

6 SCIENTIFIC RATIONALE

6.1 Introduction

This Opinion made use of the relevant scientific literature on the use of NHPs and alternatives in biomedical research worldwide. However, in the interpretation of studies it is taken into account that different jurisdictions may be involved and that the regulations with regard to NHPs can be different from those in the EU.

6.2 Ethical issues

The close phylogenetic relationship of NHPs with humans makes them the best available animal models for addressing particular research questions. Therefore, there is consensus within certain sections of the scientific community that, where alternatives do not exist, appropriate use of NHPs remains essential in some areas of biomedical and biological research and for the safety assessment of pharmaceuticals (Weatherall *et al.,* 2006). However, this similarity renders the acceptance of inducing pain or distress as a consequence of their use in scientific procedures even more debatable/challenging than for other species of animals (The Boyd Group, 2002; Weatherall *et al.,* 2006). Furthermore, animals cannot consent to their participation in research and generally will not benefit from it. Hence, their use in research represents a serious ethical dilemma, and there is a variety of views on whether NHP experiments should be permitted (Prescott, 2010). Opinion polls of the European public repeatedly show low levels of acceptance of the use of NHPs in research (European Commission, 2006; Crettaz von Roten, 2012; Clemence and Leaman, 2016).

The approach to this ethical dilemma most often adopted in regulatory frameworks on the use of animals for scientific purposes is a pragmatic, utilitarian one, which is important for protecting human safety. Utilitarianism is a branch of ethics in which the

[7]http://ec.europa.eu/health/scientific_committees/consultations/calls/scheer_call_info_01_en

moral worth of an action is determined by its outcome. It requires us to strike the most favourable balance of benefits and costs for all the sentient individuals affected by what is proposed to be done. In the case of NHP research, the human interest in potentially obtaining some benefit for mankind must be balanced against the interests of the NHPs in avoiding harm (Quigley, 2007). This case-by-case approach, referred to as "harm-benefit assessment", forms a key part of the authorisation process for projects involving animals under Directive 2010/63/EU. The EC and others have published guidance on how to perform robust harm-benefit assessments (European Commission, 2013; Home Office, 2015; Brønstad *et al.,* 2016; Laber *et al.,* 2016). However, there is some question whether the Utilitarian approach is the most appropriate one for NHP research (Rossi, 2009; Walker, 2006; Quigley, 2007).

Further recognising the high levels of public concern about NHP research, regulatory authorities in some world regions have also adopted ethical limits or boundaries on NHP use, giving rise to legislation, which is a hybrid of utilitarian and deontological ethics (the latter being based on duties and adherence to rules, rather than the consequences of actions). For example, Directive 2010/63/EU places an effective ban on the use of Great Apes in procedures, save for exceptional circumstances (subject to the use of a safeguard clause; see Article 55). For a summary of the provisions relating to NHPs in international legislation on animal use in science, see Chapman *et al.,* 2015 and Bayne and Morris, 2012.

Opinion polls of the general public show greater acceptance of animal research where animal use and suffering are minimised (e.g., Ipsos MORI, 2014) in line with the **3Rs principle,** which can be summarised as:

- Replacement of animals with non-animal methods,
- Reduction of the number of animals used to obtain information of a given amount and precision,
- Refinement of scientific procedures and husbandry to minimise harm (e.g., pain, suffering, distress) and improve animal welfare.

Definitions of the 3Rs have evolved since their original inception by Russell and Burch (1959). For example, refinement is now generally accepted to apply to all aspects of the animal's lifetime experience and to encompass promotion of positive welfare states as well as minimisation of suffering (Buchanan-Smith *et al.,* 2005). More detailed and contemporary definitions are provided in Annex 1.

Directive 2010/63/EU requires implementation of the 3Rs during the design and conduct of animal studies. However, their successful implementation relies on the proactive and coordinated engagement of multiple stakeholders, including researchers, veterinarians and animal care staff, research funders and regulatory bodies requiring animal test data. To fully apply the 3Rs and maximise the benefits, there is a need to ensure that as new knowledge, technologies and approaches emerge there is timely assessment and evolution of research strategies, study designs, scientific procedures and husbandry, throughout the lifetime of research projects.

In addition to its value as a practical ethical framework for more humane *in vivo* research, thereby helping to address societal concerns, the 3Rs concept also has considerable scientific merit (Graham and Prescott, 2015). This is because replacement is about accelerating the development and use of human-relevant tools, based on the

latest technologies, which may be more predictive of responses in man, more cost effective, and allow a higher throughput of studies (Sewell *et al.,* 2016). Approaches to reduction often focus on principles of good experimental design, and better interpretation and reporting of studies, helping to improve the quality and reproducibility of animal experiments (Parker and Browne, 2014). Animals free from unnecessary pain and distress yield better quality and more reproducible data (Poole, 1997). Hence, there are strong scientific and business drivers for the 3Rs, which increasingly are leading to changes in practice in both industry and academia (Burden *et al.,* 2015).

There is interplay between the 3Rs and conflicts can arise, such as when procedures that enable a reduction in animal numbers lead to cumulative distress for the fewer animals that are used (as might occur in a longitudinal study using imaging, for example). This conflict is usually resolved by carefully weighing the harms and benefits to the animals involved, or else by prioritising the experience of the animals (i.e., refinement) over reduction. The re-use of NHPs can decrease the number of animals used overall, however there are ethical considerations against as well as for re-use. Recital 25 of Directive 2010/63/EU states: "*The number of animals used in procedures could be reduced by performing procedures on animals more than once, where this does not detract from the scientific objective or result in poor animal welfare. However, the benefit of reusing animals should be balanced against any adverse effects on their welfare, taking into account the lifetime experience of the individual animal. As a result of this potential conflict, the reuse of animals should be considered on a case-by-case basis*." (Recital 25 of the Directive 2010/63/EU).

In the majority of cases, NHPs used in research and testing are euthanised as an integral part of the experiment for analysis of tissues post mortem. In situations where this is not required, or in the case of former breeding or surplus stock animals, it may be possible to rehome the animals as an alternative, and this is permitted under Directive 2010/63/EU. However, the rehoming process can entail welfare costs to the animals involved. NHPs should thus only be rehomed if it is clear that the process will be truly in the best interest of the individual animals, that it will not harm their welfare and that the new home offers a good quality of life (Kerwin, 2006; Prescott, 2006, NCad 2016 report: file:///C:/Users/mprescott/Downloads/ncad-opinion-rehoming-former-lab-animals.pdf).

6.3 Housing and husbandry

Europe has implemented major improvements in the housing and husbandry of laboratory NHPs over the last decade. Under Directive 63/2010/EU, it is now mandatory to house these highly social animals in socially stimulating environments, unless there are exemptions for scientific, animal welfare or animal health reasons (Article 33(3)). In addition, environmental enrichment must be provided, to allow performance of a wide range of normal behaviour. Enriched housing conditions can have a positive impact on both the psychological wellbeing of the animals and the research outcomes (Gottlieb *et al.,* 2015; Hannibal *et al.,* 2017). Improvements also include the development and implementation of training protocols designed to encourage animals to co-operative voluntarily with husbandry and scientific procedures (e.g., Prescott and Buchanan-Smith, 2003, 2007; Prescott *et al.,* 2005; Graham *et al.,* 2012; Coleman and Maier, 2010). In the past EUPRIM-Net has developed such training protocols and has disseminated them

extensively across European NHP facilities. A new EU COST Action (PRIMTRAIN[8]) is in place to develop and exchange training protocols, building on the earlier work of the EUPRIM-Net consortium. It aims to implement a minimum European standard for animal training for NHP facilities.

The early life experience of NHPs is important for their welfare later in life. Care should be taken to source animals that are weaned at an appropriate age (Prescott *et al.,* 2012) and that are well habituated to humans at the breeding centre, as this will mean that they are better able to cope with experimental use in the laboratory, especially for studies that involve frequent/close human-animal interaction (Tardiff *et al.,* 2006; Jennings and Prescott, 2009). There is the potential for breeding centres to prepare animals for their future use by training them using positive reinforcement methods to perform behaviours such as targeting shifting, and entering a transport cage. There is evidence that voluntary exposure to simple operant tasks at the breeding centre, such as pressing a button for automated delivery of food or fluid reward, can expedite learning of more complex tasks in the neuroscience laboratory, and hence provide a basis for selecting those animals likely to perform well on-study (Tulip *et al.,* 2017). The breeding unit provides a natural complexity of environment that lends itself to studies of primate welfare and behaviour.

6.4 Animal welfare standards outside the EU

Animal welfare standards for laboratory NHPs are on average higher in many European countries than in other parts of the world. There are a number of reasons for this, including differences in legislation, practice, awareness of the welfare needs of NHPs and animal health status. For example, the minimum enclosure sizes and space allowances permissible for macaques under Annex III to Directive 2010/63/EU are, depending on the age of the animals, six- to ten-times greater than the minimum permissible under the USA ILAR Guide (European Union 2010; National Research Council 2011). NHPs in Europe are more often housed in pens or customised rooms rather than small stainless steel cages with gridded floors (though some laboratories outside of Europe offer EU-style housing) (see the NC3Rs Macaque Website for examples:[9]). Housing NHPs in pairs and groups is more standard in Europe than the USA and Asia (e.g., Couch *et al.,* 2015), where Institutional Animal Care and Use Committees (IACUCs) may grant exemptions from social housing for reasons that would not generally be acceptable in most European countries. Accreditation schemes, such as the one administered by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), are valuable but do not require EU standards outside of Europe.

It follows that if NHP research is forced outside of Europe then there would likely be a net decrease in animal welfare. A decline in welfare standards need not be axiomatic in all cases, however. For example, the major UK public funders of NHP research require standards equivalent to the UK Animals (Scientific Procedures) Act and the NC3Rs guidelines 'Primate accommodation, care and use', wherever in the world the research is conducted (NC3Rs 2006; NC3Rs/AMRC/BBSRC/Defra/MRC/NERC/Wellcome Trust 2015). Compliance is assessed case-by-case for each grant application by the NC3Rs. In recent years, the European pharmaceutical industry has opened animal facilities in China and

[8]http://www.cost.eu/COST_Actions/ca/CA15131
[9]http://www.nc3rs.org.uk/macaques/captive-management/housing/

other growing economies to take advantage of emerging markets. Outsourcing of studies to the expanding contract research base in China has also increased (Xia and Gautam 2015). To maintain appropriate standards, some companies have developed global animal welfare policies that apply at all of their facilities, and most consider animal welfare issues when choosing and auditing contractors and suppliers (Underwood, 2007). These are good examples on how the commitment to actions related to international harmonisation of regulations and welfare standards, including engaging with other countries through the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the Organisation for Economic Co-operation and Development (OECD), can improve conditions abroad to bring research practices in line with European standards.

However, there are also concerns that European scientists are transferring their research programmes outside the EU to countries where welfare/scientific standards would not be judged ethically acceptable in Europe (Cryanoski, 2016; Anon, 2016). Decreased animal welfare may lead to more stressed animals and this could have an impact on the quality of the research. This may also affect public health and accessibility of treatments developed under different standards and on local economy.

6.5 Experimental design and staff training

Good experimental design, analysis and reporting are key means of maximising the knowledge gained from animal experiments and avoiding wastage of animals. With NHPs being such precious resources, it is crucial for scientific, ethical and economic reasons that their use is optimised. However, recent years have seen growing concern about the reliability and reproducibility of animal studies, and poor experimental design and reporting have been implicated as major contributing factors (see Begley and Ellis, 2012, Collins and Tabak, 2014, and other articles in the *Nature* specials archive: Challenges in irreproducible research; AMS/BBSRC/MRC/Wellcome Trust 2015, 2016). There is no reason to suspect that NHP research is any better in this regard than other fields of *in vivo* research.

For example, Kilkenny and colleagues (2009) conducted a systematic survey of the quality of reporting, experimental design and statistical analysis in 271 papers reporting research on live rats, mice and NHPs carried out in UK and US publicly funded research establishments. Only 59% of the studies stated the hypothesis or objective of the study and the number and characteristics of the animals used. Most of the papers surveyed did not report the use randomisation (87%) or blinding (86%), to reduce bias in animal selection and outcome assessment. Only 70% of the publications that used statistics described the statistical tests used and presented the results with a measure of precision or variability. These findings are by no means isolated. Poor reporting is consistently highlighted in systematic reviews of animal research (de Vries *et al.,* 2014). These are important factors in the design of studies and have serious implications for animals, science and society.

Good experimental design is one of the most effective and immediate ways to reduce and refine animal procedures, but researchers are not getting sufficient training in this essential area for completing ethical, rigorous and efficient research (Howard *et al.,* 2009). Therefore, it is essential that training in experimental design is improved and made more widely available. There have been a number of important initiatives/resources launched to aid researchers to improve the design and reporting of

research using animals. The ARRIVE Guidelines of the NC3Rs[10] (Kilkenny *et al.,* 2010) lay out the criteria that should be met in reporting animal studies in order that their results and conclusions can be properly evaluated by readers. These criteria address a range of issues relating to transparency and validity of experimental design, the avoidance or minimisation of bias, and the adequacy of statistical aspects of the study, such as power and analysis methods. The Guidelines have been endorsed by over 1,000 journals internationally, including major titles like *Nature*, *Cell* and *PLoS*, and by UK biomedical research funders, who have revised and update their peer review processes in response. The NC3Rs has also launched the Experimental Design Assistant (EDA) (https://eda.nc3rs.org.uk/) a free online resource to support researchers, particularly those in the early-stage of their career, in the planning of animal experiments. The EDA consists of a website with comprehensive guidance on experimental design and a web application which uses computer-based reasoning to provide tailored feedback and advice on individual experimental plans. The system also includes dedicated support for randomisation, blinding and sample size calculation, helping to ensuring robust study design and reliable and reproducible findings.

Appropriate training for staff working with NHPs is essential to ensure compliance with legislation, good quality science, good animal welfare and full implementation of the 3Rs. Special knowledge and practical skills are required in order to be able to address the complex behavioural, social and psychological needs of NHPs and the ethical considerations involved in their use in research (Jennings and Prescott, 2009).

Guidance to Member States on the minimum training requirements for those involved in the care and use of animals under Directive 2010/63/EU includes some species-specific learning outcomes (Anon, 2014). NHP-specific material has been provided as part of this initial, mandatory training in some Members States for many years, with some courses being accredited by the competent authority or accrediting bodies on their behalf. However, a number of issues with the current training provision in the EU need to be addressed to deliver the best science and animal welfare and to avoid perpetuation of out-dated practices and unwanted variation between laboratories.

First, NHP-specific training provided to prospective licensees is often very short (one day or less) and can only provide a very brief overview of the required information. A very short period of initial training also means that there is a particular need for Continuing Professional Development (CPD) to expand and maintain the knowledge and skills of those working with NHPs. Many institutions do not provide this on-going training, so there is a strong case for increasing the scope of both initial training courses and introduction of mandatory CPD (Weatherall *et al.,* 2006).

Second, there is variation in the syllabus content and delivery for initial training courses in Member States and no common agreed approach. A survey at the 2010 NC3Rs Primate Welfare Meeting, a key forum for CPD and networking within the EU NHP research community, found that 79% of delegates considered there was a need for an accredited course on NHP use, care and welfare. Specific gaps in existing training provision identified at the meeting were

- NHP behaviour
- Animal training

[10]http://www.nc3rs.org.uk/ARRIVE

- Surgery
- Anaesthesia and analgesia
- Welfare assessment
- Humane endpoints

In response to this, the NC3Rs organised workshops on animal training and refinement of chronic implants, and also a network for animal welfare officers working in NHP units. The training courses run from 2007 to 2015 as part of the EC-funded projects EUPRIM-Net I and II have also helped to support training in a range of areas. They take a blended learning approach and cover aspects of experimental design, imaging techniques, housing and husbandry, colony management and animal behaviour management.

Training in practical skills is another key issue. The Primate Welfare Meeting survey found only 15% of delegates believed there are sufficient practical training opportunities for those working with NHPs. This may be related to the way in which some Member States have interpreted Article 8 of the Directive, which prevents the use of NHPs for "*higher education, or training for the acquisition, maintenance or improvement of vocational skills*". However, this article should not prevent staff with the appropriate licenses gaining practical skills by undertaking, under supervision, authorised procedures required as a necessary part of on-going research projects. The problem may also be related to financial and time constraints preventing researchers from visiting other laboratories to observe and learn new techniques, for example approaches for surgical implantation of new recording, dosing or sampling devices. Although the concept of "*see one, do one, teach one*" is still considered valid within medical surgical training (Kotsis and Chung, 2013), those performing procedures in NHPs will only rarely have undergone the extensive training in surgical skills which underpins the assimilation of more complex procedure by surgeons.

New opportunities to implement the 3Rs arise continually and it is important that researchers and animal care staff keep abreast of the latest developments in NHP use, care and welfare. Published literature and scientific events provide opportunities to expand knowledge and skills. Increasingly, these traditional routes of information exchange are being complemented by dedicated online resources aimed at sharing best practice and enhancing the training and CPD of staff working with NHPs (Prescott, 2016). These include The Macaque Website[11], Common Marmoset Care website[12], Chronic Implants Wiki[13] and Experimental Design Assistant of the NC3Rs.

See Section 7 for recommendations.

6.6 Areas of research (fundamental, translational and applied) and testing of products and devices

6.6.1 Overview on the use of NHPs in research and testing

The SCHEER has used publicly available information from Member States for 2014 to provide an overview on uses of NHPs in Europe (for the sources see Annex 1).

For 2014, a total of 8898 uses (uses includes reuse of the same NHP, does not refer to

[11]http://www.nc3rs.org.uk/macaques/
[12]http://www.marmosetcare.com
[13]https://www.ciwiki.net//

the total number of animals) of NHPs were reported by European Member States (Table 1, refers to the whole EU). Species most frequently used were cynomolgus monkeys (7098, 79.7%), marmoset and tamarins (743, 8.4%) and rhesus monkeys (612, 6.9%). Importantly, these numbers are based on experimental procedures and include first use as well as any subsequent reuse of the NHPs[14]. The number of animals used in procedures could be reduced by performing procedures on animals more than once, where this does not detract from the scientific objective or result in poor animal welfare. However, the benefit of reusing animals should be balanced against any adverse effects on their welfare, taking into account the lifetime experience of the individual animal. As a result of this potential conflict, the reuse of animals should be considered on a case-by-case basis. The Directive 2010/63/EU on the protection of animals used for scientific purposes lays down basic criteria for the reuse of animals. In accordance with veterinarian advice, an animal may be reused if the previous procedure was of "mild" or "moderate" severity, the animal's state of health and well-being has been fully restored and the subsequent procedure is classified as "mild", "moderate" or "non-recovery" (Art. 16).

Table 1: Experimental procedures reported for NHP species by all European Member States in 2014

Prosimians		**203**
Cercopithecoidea (OW)	Cynomolgus monkey	7098
	Rhesus monkey	612
	Vervets, Chlorocebus spp.	19
	Baboons	183
	Other species of OW monkeys	11
Ceboidea (NW)	Marmosets and tamarins	743
	Squirrel monkey	4
	Other species of NW monkeys	25
Total uses of NHPs		8898

NW: New World species (primates of the superfamily Ceboidea, of Central and South America), OW: Old World species (primates of the superfamily Cercopithecoidea, originating from Africa, the Arabian Peninsula, and Asia).

The EU distinguishes different categories of research areas were animal experiments may be conducted, i.e., 1) basic research, 2) translational and applied research, 3) regulatory use and routine production and 4) higher education or training for the acquisition, maintenance or improvement of vocational skills. These categories are included in the forms for Member States to communicate data on use of experimental animals. Table 2 illustrates the distribution of NHP testing in relation to these categories

[14]Revised requirements for the reporting of statistical data on the use of animals in the EU in Annex II of Commission Implementing Decision 2012/707/EU http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02012D0707-20140115

which are reported by most Member States. Regulatory use and routine production is the category with the most NHPs used. With 74.5% of all NHP uses (6196) this category is of high importance regarding the application of the 3Rs.

Table 2: Distribution of NHP testing in relation to the different purposes (2014) as published by the majority of Member States having used NHPs

		Basic research	Translational and applied research	Regulatory use and Routine production	Higher education or training for the acquisition, maintenance or improvement of	Total
		(1)	(2)	(3)	(4)	
Prosimians		201	2			203
Cercopithecoidea (OW)	Cynomolgus monkey	155	744	5713	28	6640
	Rhesus monkey	233	182	101	2	480
	Vervets, Chlorocebus spp.		5	14		19
	Baboons	134	17			151
	Other species of OW monkeys	8	3			11
Ceboidea (NW)	Marmosets and tamarins	314	59	368	2	743
	Squirrel monkey	4				4
	Other species of NW monkeys	25				25
Total		**1074**	**1012**	**6196**	**32**[15]	**8314**

Additional data as well as statistical analysis on the use of NHPs like breakdowns e.g. by primary purposes, specific research area or severity of treatment vary among the Member State reports. In addition, 2014 was the first year for reporting under a new format. Therefore no further statistics could be carried out by the SCHEER to provide reliable further information or trends regarding the use of NHPs. A collation of the Member State reports on NHP use including comments on comprehensiveness of the reporting and the quality of the statistics that were published by Taylor and Rego (2016).

[15]Uses were part of projects that were authorised before 1.1.2013; 18 uses were incorrectly reported (correct category should have been regulatory use as these concerned training of the animals in regulatory procedures)

For the sake of transparency and monitoring progress in the application of the 3Rs, it would be desirable that all Member States report the same type of information and that included categories would include the level of severity of experimental procedures, specific research area, origin of animals, generation and first time use (see e.g., Home Office, Annual Statistics of Scientific Procedures on Living Animals – Great Britain, 2014).

6.6.2 Development and safety testing of pharmaceuticals and medical devices

6.6.2.1 Introduction

The focus in this section is on pharmaceuticals and medical devices because for all other products testing on NHPs is generally not permitted. In addition, the use of NHPs is only permitted in those biomedical areas essential for the benefit of human beings, for which no other alternative replacement methods are yet available. A report commissioned by several major UK funding agencies argued that evaluation of any proposed primate research project should actually consider four factors: quality and importance of the science, likelihood of medical or other public benefit, likelihood of animal suffering, and availability of alternatives (Bateson, 2011).

In 2012, the EC adopted a package of measures on innovation in health that included proposals to revise existing legislation on general medical devices and *in vitro* diagnostic medical devices. The revisions affected all kinds of medical devices. Any animal model selected should be generally accepted for the study of the device type and there should be a reasonable amount of scientific evidence that the animal model has utility for the intended study of the device type.

Currently, animal research is viewed as an essential part of the drug development process. In its report, "Working to reduce the use of animals in scientific research" (UK Government, 2014), the UK Government states: "over the past twenty years, the development of monoclonal antibody therapies has completely transformed the ability to treat diseases, including cancers, rheumatoid arthritis and multiple sclerosis, and the development of this technology would not have been possible without the use of animals, including nonhuman primates (NHPs)".

The development of a pharmaceutical is a stepwise process involving an evaluation of animal and human efficacy and safety information. The nonclinical safety testing of new medicines is conducted according to international regulatory testing requirements, frequently in accordance with guidelines agreed in meetings under the auspices of the International Council for Harmonisation (ICH), formerly known as the International Conference on Harmonisation. The ICH's mission is to make recommendations towards achieving greater harmonisation in the interpretation and application of technical guidelines and requirements for pharmaceutical product registration, thereby reducing or obviating duplication of testing carried out during the research and development of new human medicines. The goals of the nonclinical safety evaluation generally include a characterisation of toxic effects with respect to target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility. Inclusion of a non-rodent species is required in the safety assessment of pharmaceuticals, see ICH guidelines ICH M3 (R2), 2009; ICH S9, 2009 and ICH S6 (R1), 2011.

Testing the safety of medical devices, such as orthopaedic joint replacement, in animals

such as NHPs before they are introduced in human clinical trials is required for both ethical and legal reasons. Nonclinical safety testing, however, also includes many improvements in technique, *in vitro* screening of devices, and investigator understanding of study model selection. These factors all enhance the ability to predict the safety and performance of medical devices in humans, as well as reducing the number of animals required for testing. It should be noted, however, that very few medical devices, e.g., orthopaedic devices, need to be tested in NHPs.

6.6.2.2 Progress Made In the Last 10 Years

Several alternatives for animal model testing are proposed every year (see Doke and Dhawale, 2015 for a review), including the increasing use of human tissues (Holmes, Bonner and Jones, 2015; Strauss and Blinova, 2017).

Bowes *et al.* (2012) pointed out that *in vitro* pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. In addition, the utility of animal models of disease for assessing the safety of novel therapeutic modalities has become an increasingly important topic of discussion as research and development efforts focus on improving the predictive value of animal studies to support accelerated clinical development (Graham and Prescott, 2015; Cavagnaro and Silva Lima, 2016). Scientists have also been conducting extensive reviews to challenge the existing testing paradigms outlined in regulatory guidelines (Galijatovic-Idrizbegovic *et al.,* 2016).

It is also recognised that in some cases earlier access to human data can provide improved insight into human physiology/pharmacology, knowledge of drug candidate characteristics and therapeutic target relevance to disease. Streamlined early exploratory approaches can accomplish this end and involve limited human exposure, with no therapeutic intent, see ICH M3 (R2). Such studies can be used to investigate a variety of parameters such as PK, PD and other biomarkers often identified by means of *in vitro* studies, which could include PET receptor binding and displacement or other diagnostic measures. Although still needing nonclinical studies to be conducted before initiating such clinical trials, use of any of these approaches can reduce overall animal use in drug development.

There has also been considerable progress made over the last few years, both in terms of reducing the numbers of NHPs required for the safety assessment of medicinal products and in the husbandry practices (Baldrick, 2011, Beaumont *et al.,* 2011; Buckley *et al.,* 2011; Chapman *et al.,* 2012; Chapman *et al.,* 2013; van Meer and Schellekens, 2015; Backes *et al.,* 2016 and Chapman *et al.,* 2016).

The value of a second species in regulatory toxicology studies is beginning to be questioned more generally. For example, the NC3Rs and ABPI has launched an initiative to investigate whether data from one species could be sufficient for the progression of a potential new drug in human clinical trials (Monticello, 2015; Mangipudy *et al*., 2014).

The addendum to the ICH S6 (R1) guideline, which specifically deals with biotechnology derived medicines where use of NHP is widespread, was written with an aim to reduce the use of animals in accordance with the 3Rs principles. The guideline also emphasises "rodent species should be considered unless there is a scientific rationale for using non-rodents". The guideline also states that when no relevant species can be identified

because the biopharmaceutical does not interact with the orthologous target in any species, use of homologous molecules or transgenic models can be considered. In addition, it is considered to be justified to use one species for all general toxicity studies when the clinical candidate is pharmacologically active in only one species. In these cases, studies in a second species with a homologous product are not considered to add further value for risk assessment and are not recommended. This revision to the guideline is expected to lead to a decrease in NHP numbers used in this area.

The EU Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues (CHMP 2010), which came into effect on 1 December 2012, states "the conduct of toxicological studies in NHPs is usually not recommended". The background and consequence of this paradigm shift was discussed by van Aerts *et al.* (2014). A further call for the waiving of animal studies on a global basis was made in a paper by Chapman *et al.* (2016).

Over the past few decades, many improvements have been implemented in animal studies on medical devices. These improvements have effectively reduced the number of animals used, while enhancing the effectiveness of the overall assessment of nonclinical safety. The development of established and reproducible animal models to mimic the human condition has resulted in the site-specific implantation of devices in the anatomical positions in which the device would likely be used. Improved understanding of these models, relative to animal *versus* device interactions, has resulted in a more accurate assessment of the device.

6.6.2.3 Justification for NHP Use

Arguments against phasing out NHPs in safety testing of pharmaceuticals are therefore similar in many ways to those regarding using rodents for toxicity testing, i.e. incomplete knowledge of integrated body systems and pathophysiology, poor representation of pharmacokinetics by *in vitro* systems (SCHER, 2005).

Safety testing of pharmaceuticals is intended to safeguard human subjects used in clinical trials through risk assessment based on the results of all nonclinical studies, including those from animal experiments. The Declaration of Helsinki[16] is a set of ethical principles developed by the World Medical Association (WMA) for the medical community regarding human experimentation. It states that the wellbeing of the human subject should take precedence over the interests of science and society.

The ICH safety guidelines are written to ensure that duplication of studies is not required for various regions in the world. These guidelines also indicate that the nonclinical studies should be performed in "relevant species", and that pivotal studies for risk assessment of pharmaceuticals, such as the repeated dose toxicity testing, usually have to be performed in two species, one of which must be a non-rodent.

1 Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

The use of a non-rodent species for the characterisation of new medicinal products aims at limiting the uncertainty in the extrapolation process from *in vitro* and animal toxicity data to the human situation. In Europe, dogs are most frequently used as the non-rodent species, and NHPs are only used when no other species showed the same primary pharmacodynamic response. NHPs make up less than 0.1% of the animals used in research. However, they can play a key role in drug development due to their similarity to humans with regard to sensory organs, hormonal systems, reproduction, immune system *etc.*, to evaluate efficacy and safety, especially for biopharmaceutical compounds (Brennan *et al.,* 2015). Species specificity can be crucial for some new medicines. Nevertheless, the species for toxicity testing should be selected based on their similarities to humans with regard to pharmacology and pharmacokinetics, including biotransformation and in certain cases also where anatomical similarities are essential. A scientific justification for the choice of non-rodent species used is a regulatory requirement.

There still exist many "pharmaceutical gaps" for various diseases, diagnostics, and conditions, as outlined in the 2013 Report "Priority Medicines for Europe and the World" (Kaplan *et al.,* 2013). This is an update to the original 2004 report and provides a public-health-based medicines development agenda, taking into account increasing life expectancy and related population aging. Research is considered essential for medical progress, and the report outlines various key findings and recommendations. The EU is pushing "for the translation of basic research into therapies", a transition that often requires the testing of new medicines in primates, as the most relevant nonclinical species. However, restrictions on primate experiments could hinder the development of new medicines (Abbott, 2014).

For some disease indications, specific NHP models were developed which successfully mimic the disease in humans, e.g., for Graves' disease (Wang *et al.,* 2013).

The European Committee for Medicinal Products for Human Use (CHMP) has defined criteria on the demonstration of relevance of an animal species to predict human safety in their Guideline on Strategies to Identify and Mitigate Risks for First-in Human Clinical Trials with Investigational Medicinal Products (EMEA/CHMP/SWP/ 28367/07), which is currently being revised. The guideline states that scientific requirements specific to the substance include:

- Presence of the required pharmacodynamic (PD) binding site and response
- Similarity to human toxicity or pharmacokinetic (PK) profile based on *in vitro* data or prior experience with related compound(s) of the same class
- Similarity to human in aspects of anatomy or physiology of specific organ systems
- Indication for the need of an additional species to investigate a toxic effect or the effects of a significant metabolite in humans which is not produced in the original non-rodent species

The Association of British Pharmaceutical Industry (ABPI) and the UK's Home Office (2002) also gave the following specific recommendations on the selection and justification of the relevance of an animal species for safety testing:

- Use of a well characterised species may be quicker and require fewer animals
- Unknown and contradictory neurophysiological sensitivity (meant to reflect differences in suffering, harm, etc.) of the species (e.g., dog vs. pig)
- Public perception (e.g., dogs and other pets)

- Availability of new pharmaceuticals in limited quantities in the early stages of development, requiring initial assessments in animals of small body size

According to all these recommendations, NHPs should only be used when it is scientifically demonstrated that none of the other non-rodent species commonly used in safety testing is appropriate for the purpose of the study. It is also important to consider the limitations of the NHP when choosing which species to use in drug safety test. For example, a recent study raises significant concern about the scientific validity to humans of drug safety studies undertaken in NHPs. Specifically, the cynomolgus monkey has been found to be remarkably resistant to liver injury caused by paracetamol (Yu *et al.,* 2014). This finding is important for numerous reasons, including the fact that cynomolgus monkeys are the NHPs used most commonly to assess drug safety.

There are *in vitro* systems in development that could negate the need for NHPs and indeed other animals (see Replacement Possibilities section below).

The following examples illustrate areas of research, where therapeutic development and safety testing in the NHP may be preferred over that in other mammalian species and also reflect on the limitations of the model in some cases.

Menstrual Cycle: Due to the similar menstrual cycle and the anatomy and physiology of the mammary gland of NHP females and human females, NHPs (cynomolgus monkeys) are the more pertinent species in terms of the prediction of relevant reproductive effects (Buse *et al.,* 2003; Cline, 2007; Luetjens *et al.,* 2005) and are, therefore, often chosen as the non-rodent species for classes of compounds that are expected to provoke effects on the female genital organs. Recently, Buse *et al.* (2014) have provided a good review of available *in vitro* and animal models of the placenta for toxicological purposes. This includes highlighting the limitations of the cynomolgus monkey model as well as its suitability. It gives details of some NHP species, which should not be used. It also suggests other models that could and may be preferable to use in place of NHPs in given circumstances such as rodents, pigs, bovine and horses. In addition, a new study raises the possibility that a new rodent model, the spiny mouse (Nowogrodzki, 2016), could be used as a future replacement for NHP menstrual-related study (see replacement possibilities section below).

Vomiting and Nausea: NHPs are less susceptible to vomiting than dogs. Thus, pharmaceuticals with an emetic effect in the dog may be tested in the monkey (Weber, 2005). Vomiting does not only limit exposure of the pharmaceutical administered, but is also a major hurdle to accurately characterise early effects on behaviour and on the cardiovascular system. However, a review by Holmes *et al.* (2009) cautions that it should not be assumed that an NHP is necessarily the best predictor for emetic liability in humans as it can depend on the emetic challenge. The paper discusses opportunities for the replacement of animals in the study of nausea and vomiting and includes suggestions that are relevant to replacing and refining NHP studies in this area.

Blood Coagulation: The coagulation system of NHP is more similar to humans than that of any other species (Abildgaard *et al.,* 1971; Lewis, 1996) and thus, NHP are often the most suited model for humans to assess potential toxicity of coagulation factors and anti-coagulation agents.

Biopharmaceuticals: NHPs are the most appropriate animals to characterise safety of many biotechnology-derived pharmaceuticals, especially monoclonal antibodies (mAbs),

since the most relevant species for testing is selected based on species-specific aspects of the immune system. Monoclonal antibodies are highly specific to their targets and accurate prediction of 'on-target' effects requires testing in a species that shows cross-reactivity, thus frequently requiring testing in NHPs as the only species cross-reacting with humanised monoclonal antibodies (APBI-NC3Rs, 2006; van Meer *et al.,* 2015). However, an Expert Working Group convened by the National Centre for Replacement, Refinement and Reduction (NC3Rs) in the UK cautioned that:

> '*The use of the NHP plays an important role in assessing the safety of mAbs. Nevertheless, the close relatedness of the NHP to man does not necessarily guarantee that they are the most appropriate species for the development of mAbs as illustrated by the TGN1412 clinical trial in 2006 where the NHP had limited utility in detecting the cytokine storm subsequently observed in the phase 1 clinical trials. Species selection should be based on the biology of the mAb and in particular its pharmacological activity. The NHP should not be used as 'default' species, as a screen or because NHP studies have previously been conducted.*' (Chapman *et al.,* 2007, Chapman *et al.,* 2016).

The report of the NC3Rs Working Group suggests strategies for the scientific and regulatory community to implement to minimise NHP use and increase the efficiency of mAb development (See Reduction and Refinement Possibilities section below).

An ongoing challenge in the development of biopharmaceuticals is preventing immunogenicity, which can lead to side effects and more commonly an immune response that causes loss of efficacy as the body develops neutralising or clearing antidrug antibodies (van Meer *et al.,* 2015). Although NHPs are often the only relevant animal model for the development of mAbs, van Meer and colleagues' study (van Meer *et al.,* 2015) suggests that the immunogenic response in NHPs is poorly predictive of the response in humans. This finding strengthens the need to develop innovative alternative models to ensure progress in this important therapeutic research area.

Central nervous system (CNS) pharmaceuticals: Assessing novel CNS pharmaceuticals may further increase the need for testing in NHPs (Vuillemenot *et al.,* 2016). While the rat is in principle acceptable for self-administration studies in the EU, NHPs are preferred in Japan.

Reproductive Toxicity: Historically, non-primate species have been used for reproductive toxicity studies, generally mice, rats and rabbits. However, rodents and rabbits are not necessarily the most accurate predictor of teratogenicity or reproductive toxicity in humans due to differences in placental anatomy and number of fetuses. In addition, they are not suitable models for all aspects of human reproductive toxicity, specifically for the investigation of agents suspected or known to interfere with the menstrual cycle. In such cases, NHPs may be more predictive for human toxicity. The male cynomolgus is also a good model of male fertility in specific cases (Ehmcke *et al.,* 2006; Millar *et al.,* 2000). Rodents can also not be used to assess the safety of novel hormonal intrauterine devices or cognitive dysfunction associated with the menopause (Schlatt *et al.,* 2008; Wistuba and Schlatt, 2002). However, there is a strong impetus within the scientific community to move away from animal-based reproductive and developmental toxicity testing (Stallman Brown *et al.,* 2012). This has resulted in progress in the development of non-animal alternatives to move toward a predictive mechanism-based approach.

Other challenges for the development of new medicines are the novelty of the molecules that are currently under development, the unique delivery systems, use of alternative routes of administration, long half-life and mismatch between pharmacokinetics and pharmacodynamics.

6.6.2.4 Replacement possibilities

Replacing NHP models in new medicine development and safety testing has the potential to significantly reduce the number of NHPs used in European laboratories, as regulatory use and routine production are consistently the areas of greatest NHP use, as discussed above. Substantial progress has been made in the development of new *in vitro* and *in silico* methods and integrated testing strategies have been proposed by OECD[17] and ECVAM[18] which have the potential to reduce, refine and ideally replace the need for NHPs and indeed other animal studies in the near future, based on the identification of AOPs (Adverse Outcome Pathways) through the integration of Mode of Action, kinetics and dynamics. The examples given include substituting NHPs for other species, which is not without ethical considerations, but can be viewed in the context of progressing toward the ideal position of complete replacement with non-animal alternatives.

Liver Injury and Testing for Idiosyncratic Adverse Drug Reactions (IADRs) in Humans: Thompson *et al.,* (2012) evaluated an *in vitro* approach, which explored both cellular effects and covalent binding to assess IADR risks for drug candidates using 36 drugs which caused different patterns and severities of IADRs in humans. They propose that this integrated *in vitro* panel of five assays approach has the potential to enable selection of drug candidates with reduced propensity to cause IADRs in humans. Shoda *et al.,* (2014) describe the DILIsym® software, a mechanistic model of drug-induced liver injury (DILI) and how its development will improve the rationale design of new drugs. DILIsym® simulates the mechanistic interactions and events from compound administration through the progression of liver injury and regeneration. Modelling mitochondrial toxicity illustrates the type and use of *in vitro* data to represent biological interactions, as well as insights on key differences between *in vitro* and *in vivo* conditions.

Menstrual Cycle and Associated Disorders: Advances in research relating to menstruation and associated disorders such as endometriosis and pre-menstrual syndrome, have been hampered by the lack of an appropriate model. The ideal model would be a small species with a short reproductive cycle and similar placental form, but traditionally the species most resembling humans has been cynomolgus monkeys (Buse *et al.,* 2014). However, very recently evidence that the spiny mouse (*Acomys cahirinus*) is the first rodent species known to menstruate indicates that it could provide an unprecedented natural non-NHP model to study the mechanisms of menstrual shredding and repair and may be useful in further understanding human-specific menstrual and pregnancy associated disease (Bellofiore *et al.,* 2016). This is new research and further studies will be required to check the validity of this rodent as a model for the human condition, but it does hold great promise as a future alternative to NHPs.

[17]http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282015%2922&doclanguage=en
[18]http://publications.jrc.ec.europa.eu/repository/bitstream/JRC96418/eurl%20ecvam%20toxicokinetics%20strategy.pdf

Swine Models: There is an expanding body of research that supports the use of swine models (especially mini-pig strains) as an alternative to the use of dogs and NHPs as the choice of non-rodent species in nonclinical toxicology testing of pharmaceuticals. Swine models share similarities in anatomical and physiological characteristics with humans such as the cardiovascular, urinary, integumentary and digestive systems (Swindle *et al.,* 2012). The pig has been particularly useful in treatment of atherosclerosis and myocardial studies. The pig and mini-pig are increasingly being used as toxicological models in safety assessment for reproductive toxicology, development of paediatric pharmaceuticals and routine toxicology (Swindle *et al.,* 2012).

In addition, Forster *et al.,* (2010) concluded that the mini-pig is well placed to meet the challenges of emerging technologies and the toxicology of the future due to the sequence homology between pigs and humans, the potential to perform toxicogenomic studies, and because the mini-pig is the only non-rodent toxicology model where transgenic animals can be readily generated and where reproductive technologies are well developed. Ellegaard *et al.,* 2010 concluded that it is easier to keep mini-pigs to a good standard of welfare under laboratory conditions than it is for NHPs or dogs.

6.6.2.5 Reduction and refinement possibilities

Where replacement of NHPs is not yet possible, there are several opportunities to reduce the numbers required in this area of research and to refine the procedures involved, which are outlined here.

Biopharmaceuticals*:* To investigate evidence-based opportunities to minimise the use of NHPs in the development of therapeutic monoclonal antibodies, an expert working group was convened by the NC3Rs. The report of the working group (Chapman *et al.,* 2007) and subsequent papers (e.g., Chapman *et al.,* 2012, 2016) provide detailed strategies and approaches that can be adopted to ensure that NHP studies are minimised and when they are necessary are efficient and effective, such as using homologous proteins and appropriate genetically altered rodents, and designing the programme of work to optimise the study duration, dose regimes and number of groups. The efficient study designs identified by the NC3Rs-industry data sharing enable the number of NHPs per mAb in development to be reduced by half. Other opportunities for reduction and refinement identified by the NC3Rs working in collaboration with industry and regulatory bodies internationally include: avoiding the use of NHPs for candidate selection (Lave *et al.,* 2009, Beaumont *et al.,* 2011), reducing the number of recovery animals on toxicology studies (Sewell *et al.,* 2014), group housing during cardiovascular telemetry recordings on toxicology and safety pharmacology studies (Prior *et al.,* 2016), reducing the upper limits of body weight loss in short term toxicology studies (Chapman *et al.,* 2013), and use of micro-samples to assess drug exposure in blood, plasma and/or serum (Chapman *et al.,* 2014a,b).

Microdosing: New applications of human microdosing studies to areas beyond exploratory pharmacodynamics and biomarkers, which could include PET receptor binding and displacement or other diagnostic measures data, could potentially reduce the number of unsuccessful candidate drugs from progressing into the full developmental process and as a consequence reduce the number of nonclinical animal tests. Lappin *et al.,* 2013 and Burt *et al.,* 2016 describe how the microdosing technique has been extended to assess the magnitude of potential drug-drug interactions and early indications of metabolic profiling of a drug. The latter could lead to improved prediction

of drug clearance in humans. Therefore, there is greater potential for the so far limited application of microdosing to be improved and its utility widely expanded to the benefit of animal models, which could include NHPs.

Patient derived induced pluripotent stem cells (iPSCs): Recently, Giri and Bader (2015) argued that iPSCs can be used as an *in vitro* clinical trial and as a consequence can provide an opportunity within the drug development process to assess human disease pathology without the need for animal models. Giri and Bader (2015) discuss the utility of iPSC methods, the technical challenges and how they can be further developed including comments on the infrastructure needed to ensure widespread use. iPSC technology enables exploration of disease mechanisms of normal healthy and diseased cells *in vitro.* This provides clues to novel therapeutic molecular targets that are directly relevant to humans. Once the targets have been identified, iPSCs can be used to screen potential drug candidates to predict their effectiveness in terms of toxicity, dosage and individual human susceptibility. Giri and Bader (2015) also note that such technology could be used to modify the conventional drug discovery process to ensure improved relevance to human disease and to produce high-quality efficacious drugs. Therefore, iPSCs have the potential to reduce the number of animal-based preclinical tests and even replace some of them.

6.6.2.6 Identification of specific research areas

This section provides information on areas of research that have the potential to require increased NHP use in the future or where there is great potential to develop alternative models and methods to replace current NHP use.

Debilitating Disease: There are a number of examples for major new treatment options for debilitating diseases where NHPs have been used in the safety assessment as the best available model for humans due to close similarities in physiology and anatomy that can be found in the European Public Assessment Reports (EPARs) on the European Medicines Agency (EMA) website[19]. This is probably a reflection of an increased number of biotechnology-derived medicines being developed for indications such as rheumatoid arthritis and cancer. Biotechnology derived medicines have tended to use NHPs as a default non-rodent species, although this is being questioned (Baumann *et al.,* 2014; Backes *et al.,* 2016).

Cognitive Processes: Brain disorders are among the largest cause of disease burden globally, affecting millions of people and imposing enormous societal and economic costs. Many of these disorders are chronic and incurable conditions for which existing treatment options are inadequate and in some cases almost completely ineffective. One specific challenge in modelling brain disorders is that many of the clinical symptoms involve higher cognitive functions that are controlled by the prefrontal cortex (PFC), which is much less developed in rodents than in primates (Kaas, 2013) and thus, NHPs are generally considered the most suitable experimental animals for research on higher-level cognitive and behavioural processes (Giarola, 2015).

Abuse Potential: New drugs must be evaluated for "actual or relative potential for abuse" (CHMP, 2006; FDA 2010). Abuse potential can be determined in multiple ways, most frequently using drug discrimination and drug self-administration. Animals,

[19]http://www.ema.europa.eu

primarily rats and monkeys, self-administer most of the drugs humans abuse and, in many cases, the use of rodent and monkey models provides similar information. While the rat is, in principle, acceptable for self-administration studies in the EU, NHPs are preferred in Japan. Working primarily with Pfizer, the NC3Rs built a case to use the rat, supporting a change in ICH M3 to accept rodent instead of NHP data (O'Connor *et al.,* 2011).

There are cases, however, when species differences emerge and it is advantageous to use NHPs for these assessments. There are differences in brain anatomy between rodents and primates (including NHPs) that make NHPs more suitable for translational longitudinal neuroimaging studies (Howell and Murnane, 2011; Murnane and Howell, 2011; Nader and Banks, 2014). As well as potential for abuse of new drugs, the regulatory uncertainty in industry about the testing and safety of e-cigarettes could lead to the use of NHPs in inhalation and addiction studies, but Combes and Balls, (2015a; 2015b) present an extensive assessment of how these new tobacco-related products could be tested using mainly cutting edge *in vitro* techniques and human clinical studies.

Prenatal Drug Exposure: Another advantage of the use of NHPs in new medicine development includes the study of prenatal drug exposure (Gill *et al.,* 2012; Soto *et al.,* 2012). Macaques develop at a similar rate to humans during the embryonic period, but at an accelerated rate thereafter. Skeletal growth and maturation is a continuous process that occurs during the pre and postnatal period through to adult. Therefore, disruptions in this process at any time during development could potentially affect growth. However, disruptions in the prenatal and early postnatal periods are likely to have the most notable effects since this is when the skeletal structures are being formed and the ossification centres appear. The choice of species is, however, driven by the same considerations as for general safety assessment in that only relevant species should be used, meaning that NHPs are used most often for biotechnology derived medicines.

Paediatric Medicine: Guidance documents on the safety assessment of medicines for a paediatric population are available (FDA 2006, CHMP 2008). A harmonised ICH guideline on the Nonclinical Safety Testing in Support of Development of Paediatric Medicines (ICH S11) is currently in development. There is agreement that juvenile toxicity testing should be considered if the available nonclinical and clinical data are not sufficiently comprehensive to support the intended paediatric clinical trials. This has led, in some instances, for the conduct of safety assessment in juvenile animals across several species. Mostly these studies are performed using rodent models (rats and mice), but in a number of cases, NHP models have also been used. For those organs that continue to develop post-birth, the most rapid maturation period is during the first 3 to 6 months in macaques and this corresponds developmentally to the first 2 to 8 years in humans. The choice of species is basically driven by the same considerations as for general safety assessment in that only relevant species should be used (Baldrick 2013, Barrow *et al.,* 2013).

The timing of the various postnatal phases is obviously different across species (rodents, dog, mini-pig, NHPs) and these differences should be considered for study planning and/if the test item targets a particular organ system (Baldrick, 2013). For NHPs, there is a lack of accepted and consistent definition of the various postnatal development phases. However, efforts have been made to provide this information for the cynomolgus monkey (Morford *et al.,* 2011; Weinbauer *et al.,* 2011).

Vomiting and Nausea: Holmes *et al.* (2009) note that the use of animal models, including NHPs, for establishing emetic liability is an issue for discussion as robust data on their predictive value are lacking. They argue that there are scientific and ethical drivers for applying 3Rs to this area. They suggest that alternatives to animal models in nausea and vomiting research have not been fully explored due to the complexity and multisystem reflexes involved. The paper calls for experts to come together and examine opportunities for replacement and to inspire innovation in 3Rs implementation in this field of research. Some of the key avenues of research that they suggest should be explored are;

- Invertebrates and other lower organisms – have the potential to act as screening to identify emetic potential early in the drug development process. Assessing the utility of these models is potentially quick and validating them against existing clinical and pre-clinical data will be essential.
- Pharmacogenomics – microarray based gene expression profiling could offer researchers the opportunity to identify emetic liability of novel compounds *in vitro*.
- *In vitro* approaches – tissue models of enteroendocrine cells from the gastrointestinal tract developed for investigating neurotransmitter responses to mechanical forces and dietary components could potentially be applied to nausea and vomiting. Further, techniques such as isolated abdominal vagal grease gap preparation could be used to assess emetic liability.

Holmes *et al.,* (2009) also provide a useful stepwise strategy incorporating these approaches that will help to reduce and refine animal use, including NHPs, in this area.

Reproductive Toxicity: There has been progress in a move toward a non-animal predictive mechanism-based approach but there are still obstacles to overcome in terms of regulatory acceptance and scientific validity. This is an area where resources and collaborative effort should be focused as there is significant scope for replacing NHPs and other animals. A report (Stallman Brown *et al.,* 2012) of a 2012 FDA Workshop to discuss emerging *in vitro* tools for predicting reproductive and developmental toxicity focuses on the ability of *in vitro* test methods to predict *in vivo* outcomes and the potential to incorporate these new methods into regulatory decision making. The report highlighted emerging methods such as whole embryo culture and embryonic stem cell tests, zebrafish and metabolomics. It also highlights several collaborative efforts to develop and validate *in vitro* assays and what strategies are need to apply such assays to regulatory decision making.

In addition, when considering toxicity testing more generally the recent findings of Bailey *et al.* (2015) indicate that tests results inferring an absence of toxicity provide no evidential weight of an absence of toxicity in another species even when data from NHPs and humans are compared. The paper strongly argues that animal tests including those involving NHPs have very little value in predicting human toxicity and that human-focused alternatives need to be adopted.

Transgenic Techniques: Transgenic macaques were first reported 15 years ago (Chan *et al.,* 2001) and an overexpression model of Huntington's disease was described in 2008 (Yang *et al.,* 2008). Despite these advances, however, the widespread adoption of transgenic primate models appeared impractical until recently, given the paucity of methods for making precise genetic changes in primate embryos. Recent developments

in transgenic techniques such as CRISPR/Cas9 and TALENS have now led to calls for the creation of lines of transgenic NHPs and arguments for their necessity to biomedical science (Jennings *et al.,* 2016*;* Scaduto, 2016).

Basic and translational neuroscience has made, and will continue to make, great progress by studying mice and other simpler organisms. Much can also be learned by studying humans directly, and technological advances in areas such as neuroimaging, genomics and induced pluripotent stem (iPSC) cells are allowing human disease researchers to address questions that were previously restricted to experimental animals. NHP research will in no way replace these approaches, and, for both ethical and practical reasons, primate genetic models should only be considered where other alternatives are not available.

In addition, this is an area of research where some argue that the moral and welfare arguments vastly outweigh the potential benefits of any such use (Coors *et al.,* 2010; Combes and Balls, 2014; Bateson and Ragan, 2014). It is therefore important to carefully consider when, why and how such projects should be attempted.

When considering these ethical issues, it is useful to distinguish between different types of transgenic studies. Many questions about brain function can be addressed in mouse transgenic knock-in lines expressing genetically encoded reporters or effectors under the control of an endogenous promoter, allowing monitoring and manipulation of activity in specific subsets of cells. The development of primate genetic models, if deemed essential, will be an international effort, involving many countries with different cultural traditions and public attitudes toward animal research. It will be important to establish shared standards and regulations and to assure all stakeholders that work is performed to the highest standards of animal welfare regardless of where it is conducted.

Finally, there is an ethical obligation to use animal resources wisely, minimising unnecessary duplication of effort and maximising the benefit obtained from each animal by sharing data and (where applicable) cell and tissue samples. This applies to all animal research, but it is especially so for NHP research given the high costs, the long lead times and the need to minimise the numbers of animals used. Achieving this will require coordination at national and international levels.

6.6.3 Treatment and prevention of infectious diseases

6.6.3.1 Introduction

Despite the development of various advanced *in vitro*, *in vivo* and *ex vivo* techniques, the pathophysiological aspects responsible for morbidity and mortality of many infectious diseases are still largely unknown. Therefore, the use of live models to study biological aspects of infectious diseases and to develop new therapies and vaccines remains indispensable. By definition, the most predictive model to study a disease is in the target species itself. However, safety and ethical barriers to study infectious diseases in humans limit the use of most human challenge models and/or patients. In general, clinicians only see patients when the disease is in an advanced stage and treatment is required. Although important data can be obtained from patient studies, both practical and ethical issues limit the in depth analysis of host-pathogen interactions. Animal studies are, therefore, important to study certain aspects of infectious diseases in depth. Furthermore, it is important that these models mimic the various responses in humans as much as possible including immunological and pathological responses. Such models

allow studies on safety, immunogenicity and efficacy of new vaccines and/or drugs and also provide possibilities for relevant research on host-pathogen interactions and the interplay with the host immune system. In some cases, NHPs provide the best, or even only, models to study these aspects in infectious disease research. Over the last decades, NHPs have been instrumental in gaining an understanding of the pathogenesis of various infectious diseases and have provided relevant models to develop new therapies, e.g., development of vaccines against polio, yellow fever, Hepatitis B and Ebola, identification of the causative agents of infectious diseases such as SARS, typhoid fever and mumps, and more in-depth understanding of infections such as HIV (e.g., Cong *et al.,* 2016; Curtis *et al.,* 2015; Fouchier *et al.,* 2003; Garcia-Tellez *et al.,* 2016; Kaushal *et al.,* 2012; Martinez *et al.,* 2015; Mason *et al.,* 1973; Prince and Brotman, 2001; Racaniello, 2006; Rivera-Hernandez *et al.,* 2014; Shedlock *et al.,* 2009, Pena and Ho, 2015; Scanga and Flynn, 2014).

6.6.3.2 Justification for the use of NHP in Infectious Disease Research

The selection of an animal model in infectious disease research depends on the specific scientific questions. Macaques, Syrian gold hamsters and mice may be used to study yellow fever and are associated advantages and disadvantages (Julander, 2016). In contrast to the other models, however, macaque models have a disease pattern similar to humans and clinical isolates can be used. Therefore, NHP models are used to study the whole array of disease manifestations and optimal protection, which is the case for a variety of other infectious diseases. To select NHPs as an animal model, the course of the disease should: 1) resemble human disease with a comparable host range of cells, organs or tissues involved and 2) reflect the host response in humans as much as possible. Additional reasons for selecting NHPs include 1) the target of a potential drug or vaccine is only present in NHPs and humans ('t Hart, 2015) or 2) NHPs are natural hosts for the pathogen (Ploquin *et al.,* 2016).

6.6.3.3 Progress made in the last 10 years

During the past 10 years, there have been many developments in the infectious disease and vaccine research field. There have been striking improvements in in-depth analysis of various aspects during infections with the aim to select biomarkers and/or vaccine candidates. Development of –omics technologies such as genomics and proteomics, and new cell culture techniques and development of organoïds have helped the downselection of vaccine candidates for preclinical studies. Rapidly developing imaging techniques and telemetry in infectious disease research provide more data from animal studies and thus, reduce animal numbers in experiments. Human challenge models and clinical data are additional developments in infectious disease and therapy research as well as the development of humanised mice and non-mammal animal models. Taken together, new approaches lead to reduction, refinement and replacement of NHP models. One major advance has been the full replacement of NHPs for testing of the neurovirulence of polio vaccines (Dragunsky *et al.,* 2003).

Replacement

For replacement, controlled human challenge models were developed and implemented for typhoid, *Plasmodium falciparum* malaria (Pollard. *et al.,* 2012) and transmission studies with specific influenza strains (Killingley *et al.,* 2012) as well as the human

attenuated TB challenge model, which is currently in development for vaccine development (Kaufmann *et al.,* 2016). However, with ethical and safety barriers preventing human challenge studies with virulent pathogens e.g., HIV, TB and Zika virus, NHP models remain in use. Moreover, because many aspects of certain infections are mimicked in NHPs, NHPs remain suitable research models (Mothé, 2015; Capuano, 2003; Garcia-Tellez *et al.,* 2016; Dudley *et al.,* 2016). Furthermore, validated correlates of protection against most of these pathogens have not been identified, which prevents the assessment of new vaccine efficacy in humans without challenge studies.

Replacement and Reduction

In vitro modelling: Recent developments in biomedical research have improved the selection of 'most promising candidates' for new therapies *in vitro* before further assessment *in vivo*. A recent example is a cell culture technique to study drugs against dormant stages of specific malaria species that substantially reduced the number of NHP experiments (Dembele, 2014; Zeeman, 2016). *In vitro*/*ex vivo* models, -omics technologies and systems biology (*in silico* modelling) provide important new opportunities for understanding host response to pathogenic organisms, e.g., growth inhibition assays for *M. tuberculosis* (Zelmer *et al.,* 2016) and can provide information on important components of pathogens for the selection of best vaccine candidates. Promising new technology, e.g., growing organoïds from specific stem cells and improved 3D cell culture techniques and techniques such as MIMIC may also provide new insights into infectious diseases and/or development of new therapies (Dauner *et al.,* 2017). Collectively, new methods may downselect new vaccine candidates and drug targets. However, these alternative methods may only provide partial information on the complex interaction between host and pathogen, though there is hope for the near future, with the development of fully artificial whole-body models.

Non-NHP animal models: The development of non-NHP animal models, such as transgenic and humanised mice or zebrafish may eventually reduce NHP use (Holz *et al.,* 2016; Arainga *et al.,* 2016; Neely, 2017, Ibeh *et al*., 2016). Unfortunately, data may be difficult to interpret due to variations in immune systems between certain non-NHP hosts and humans (Garcia-Tellez, 2016). Additionally, there may be the need to adapt the pathogen to the host, e.g., influenza or Dengue virus in mice, which is less problematic with NHPs. Currently, there are very few non-NHP models available that have the capacity to fully replace NHP models in the near future.

Refinement and Reduction

Imaging techniques: Imaging techniques, such as PET-CT scanning or fluorescent imaging within infectious disease may enable the study of the development of certain infections, including the early effects of infection, and/or therapy over time in the same individual (Lin *et al.,* 2016). Novel sophisticated imaging techniques including new tracers and labelled mAbs will enable detailed analysis of the development of infections in a single animal, which will increase the amount of information from fewer animals (Bocan *et al.,* 2015).

6.6.3.4 Identification of specific research areas

The (re)emergence of infectious diseases over the past years demonstrates the need for research and new therapies. A recent example is the re-emergence of whooping cough caused by *Bordetella pertussis* for which small animal models are not reliable for vaccine

development. However, a new baboon model was developed that mimics whooping cough in patients (Warfel *et al.,* 2013). Important diseases such as TB and simian immunodeficiency virus (primate analogue of HIV) are more human-like in NHPs than in other animal models. Human challenge models are currently not possible because of the potential chronic nature of the infection and absence of relevant non-NHP models for the specific research question. *Plasmodium vivax,* one of the most prevalent malaria infections in the world, can only be partially studied in humanised mice (Mikolajczak *et al.,* 2015). Additionally, Macaques infected with a macaque-specific analogous parasite may only provide limited information on *Plasmodium vivax* infection (Joyner *et al.,* 2015). Although NHPs are important the study of malaria infection and therapy, NHP models also have their limitations (Martinelli, 2016). Depending on the question, malaria liver stage research may be studied in mouse models (Holz *et al.,* 2016). There are several studies stating that there is a lack of available vaccines, even after many years of research in NHP models for HIV research (Akhtar, 2015; Bailey, 2014). However, NHPs have been essential for improving our understanding of HIV and in the development of new interventions that resulted in a longer survival of HIV patients. Recently, a specific NHP HIV model was found to have many similarities with disease development in HIV-infected children with non-progressive disease (Muenchhoff *et al.,* 2016). NHP models are thus, indispensable for the study of the fundamental aspects of HIV and development of prophylactic and therapeutic therapies.

Changes in spread, infectivity and pathogenicity of infectious agents are realistic dangers. Examples are recent Ebola and Zika epidemics as well as new pandemic influenza strains. Because of many years of NHP research on Ebola, vaccines were available relatively quickly and could be tested in endemic areas. Zika was originally discovered in NHPs in the 1940s and currently, Zika infections are studied in NHPs. Dengue virus, the most prevalent arbo-virus with potential severe morbidity, does not naturally infect non-human species. However, in contrast to all other immune-competent animal species, Dengue infects and replicates in NHPs and stimulates relevant immune responses (Clark *et al.,* 2013). Thus, NHPs provide essential models for understanding and combatting (re)emerging infectious pathogens.

Infectious disease models are better in NHPs than in other animals when the disease resembles that in humans compared with other animal models. A major advantage is the similarity of NHP and human immune systems. Most reagents for analysis of human immune responses can be reliably used in NHPs. Additionally, increasing knowledge of the NHP major histocompatibility complex (MHC) genes has substantially improved the understanding of HIV and will have an impact on other diseases, such as emerging viral infections, TB and malaria, which will also be essential for elucidating mechanisms underlying host immune control of pathogens. Because genetics between humans and other species, including NHPs are not identical, small differences may influence the outcome of an infectious disease or therapy (Bailey, 2014). Differences between humans and NHP in their innate response to LPS have been reported (Barreiro, 2010), which can be used both as weaknesses or strengths for NHP models in this field.

Due to their phylogenetic relationship, the MHC of NHPs and humans are more closely related than that of other animal species and as a result often offer the best fit-for-purpose models. Small differences in genetics between different species (e.g., rhesus versus cynomolgus macaques) or even within species derived from different geographical origins in combination with different disease outcomes (e.g., HIV and TB)

provide the opportunity to study fundamental mechanisms underlying pathogenesis and host response ('t Hart *et al.,* 2015; Sharpe *et al.,* 2016).

Future Research

Further optimisation and characterisation of non-NHP animals, such as humanised or transgenic mice and zebrafish, can reduce the use of NHP models for specific questions. Further development of new techniques such as organoïds and -omics technology, 3-dimensional cell culture techniques and systems biology for vaccine development will reduce the current necessity to use NHP to study important infections[20]. Development of human challenge models should be encouraged when possible. Platforms for collaboration and sharing of data from population studies, genome wide association studies and clinical studies should be strengthened to further advance knowledge and thus development of new therapies and vaccines. The use of imaging techniques in both preclinical and clinical studies are important to reduce or replace NHP models and will be helpful to compare NHP and patient data. Improvement and development of new imaging techniques, tracers (e.g., to allow PET imaging) and labelled antibodies are necessary to follow infections *in vivo* in individual animals. Research leading to the identification of predictive biomarkers for vaccine efficacy in animal studies, including NHP, and patients will result in the possibility to perform better predictive vaccine efficacy studies in humans without requirement of a pathogen challenge. Reduction and refinement of NHP models in infectious disease research can also be achieved by further and better characterisation of NHP. Examples include the in-depth characterisation of the genetic background, which will improve the understanding of disease, therapy, improved animal selection (Haus *et al.,* 2014; Vierboom *et al.,* 2016) and characterisation of the microbiome. Additional refinement will be reached by continuously improving animal training protocols to reduce potential stress responses that can influence the outcome of the research. Sharing of biological material obtained from NHPs, e.g., through biobanks, should be encouraged to further reduce the number of animals used. In some cases, the use of NHPs is mandatory to test a new vaccine master or working seed lots, such as for yellow fever (WHO Technical Report Series, No 872, 1998). Replacement of live vaccines that have a risk for neurological effects and were master or working seed lots are now tested in NHPs by subunit vaccines. Currently, it is not possible to provide a fixed time-schedule for reducing the number of NHPs used in infectious disease research. It is unlikely that the new technologies will fully replace NHPs in the near future, especially with the (re)emergence of pathogens. Moreover, this will also depend on potential outbreaks with novel pathogens in which NHPs are the most relevant or possibly, the only reliable model species.

6.6.4 Neuroscience

6.6.4.1 Introduction

Historically, NHPs are used as models of the human brain in fundamental research to elucidate how brain circuits contribute to memory, attention, decision making, reward, emotion, visual and auditory perception and motor control (Bystron *et al.,* 2006; Dehay and Kennedy, 2007; Garcia-Cabezas *et al.,* 2008; Letinic *et al.,* 2002; Meyer *et al.,* 2000; Georgopoulos, 2000; Sanchez-Gonzalez *et al.,* 2005; Smart *et al.,* 2002; Schultz,

[20]http://www.iprove-roadmap.eu

2002; Fabbri-Destro and Rizzolatti, 2008; Rizzolatti and Fabbri-Destro, 2008). Although the number of NHPs used per study is typically small, many neuroscience research groups within Europe are engaged in this type of academic research. The continuum between such fundamental primate research and applied neuroscience research in patients remains the key to advances in the field.

In addition to advancing scientific knowledge about the brain, information gained from fundamental research on NHPs has helped to understand brain dysfunction and has sometimes contributed to the identification of treatments against disease and injury in patients. For example, behavioural techniques to study the consequences of brain injury in NHPs have been translated to allow assessment of human patients. Changes in learning after lesions of different regions within the frontal lobe (Kennerley *et al.,* 2006, Rudebeck *et al.,* 2008) were used by another research team to elucidate the difficulties of human patients with frontal lobe lesions (Camille *et al.,* 2011). Understanding the consequences of brain lesions in NHPs had a direct bearing on understanding the impairment of patients with lesions that included parts of the same brain areas. The clinical work could not have been done without the initial animal studies. Fundamental neuroscience research has also helped, along with data from rodents and man, construct new experimental *in silico* and *in vitro* models and to develop new computational technologies to simulate how the brain works. An ongoing example of this endeavour is the Human Brain Project[21]. These replacement techniques partially rely on electrophysiological data acquired in animals. Substantial work in the field of imaging techniques has been achieved due to the work with NHPs in fundamental neuroscience research (Logothetis, 2001). Electrophysiology studies in the awake, behaving state are generally assessed as imposing a high welfare impact due to the number of procedures involved (e.g., surgeries under anaesthesia, chronic restraint and penetration of microelectrodes, food or fluid control), their likely effects on the monkeys, their repetition over many months or years, and long-term housing in the laboratory environment (Bateson *et al.,* 2011) (also see Severity Classification below).

Translational neuroscience research aims at understanding the causes of, and developing new therapies against, a range of brain disorders like Parkinson's disease, Alzheimer's disease or multiple sclerosis. These disorders remain poorly understood and have an important impact on society in terms of number of patients (510 million in the EU in 2015) and their affected relatives, as well as on public health cost (WHO NeuroAtlas, EBC). Multiple strategies should be deployed to advance knowledge in this area. Whilst rodents are used for this research, applied and translational research in NHPs can be key in some steps of the process because of their greater similarity to man. Both fundamental and applied studies can show discrepancies between primates and non-primate species, thus avoiding the pursuit of certain research directions that could fail if translated to the clinic.

Modelling neurodegenerative diseases in NHPs can cause a high degree of suffering to the animals. For example, the MPTP model of Parkinson's disease can result in severe impairment of the well-being and condition of the animals, necessitating daily examination, warming, weighing, hand feeding and grooming until they regain autonomy (Jackson and Jenner, 2012). In the chronic phase, motor symptoms of Parkinson's disease closely resemble the pathology in patients, which includes abnormalities in gait,

[21]https://www.humanbrainproject.eu/2016-overview

balance and posture, slowness of movement, freezing, rigidity and levodopa-induced dyskinesias. The caging environment needs to be adapted to these symptoms (reduced height of elevated platforms in the cage, use of grids instead of bars, plastic instead of metal grids to avoid contact with cold surfaces on platforms and cage flooring, adjusting the height of food and beverage receptacles to grant ease of access, etc.) and to minimise risk of injury. Alternatively, other NHP models of neurodegenerative disease like 3NP systemic intoxication or quinolinic acid intra-striatal lesions for Huntington's Disease, overexpression of mutant proteins like huntingtin (for Huntington's Disease), alpha-synuclein (for Parkinson's Disease), amyloid or Tau (for Alzheimer's Disease), are mostly asymptomatic and currently classified as moderate since they only require one surgical intervention that does not induce long lasting suffering throughout the lifespan of the animal.

However, given the potential for serious adverse effects of some models and techniques and the long-lasting nature of some of these studies, it is particularly important that funders and ethics committees establish robust peer review procedures to ensure that only those projects with a very high likelihood of producing scientific, medical or social benefit go ahead, and that there is full application of the available opportunities to implement all three Rs.

6.6.4.2 Severity classification

Directive 2010/63/EU requires that severity classification take into account, among other things, the lifetime experience of the animals, the duration, frequency and multiplicity of harmful techniques, the potential for cumulative suffering within a procedure, and the application of refinement techniques (see Annex VIII). Prospective severity classification for the purposes of project evaluation is done for the entire group of animals undergoing the procedure/s, and is based on the most severe effects likely to be experienced by any individual animal after applying all appropriate refinement techniques. On the other hand, retrospective reporting of actual severity (e.g. for annual statistical reporting) is based on the actual experience of each individual animal. Guidance on severity assessment from national competent authorities[22] states "The actual severity to be reported for the individual animal should be the highest level experienced during the course of the procedure and not based on the severity at the end of the procedure. Nor should the evaluation be considered a simple additive process, e.g., a number of mild procedures = moderate severity. It should be based on an overall assessment of the animal's experience from the start of the procedure to the end." Permission to re-use is dependent on actual severities of the previous procedure/s in combination with prospective classification of proposed re-use. Reuse permission is not based on prospective classification of the previous procedure.

Annex VIII of the Directive gives examples of procedures under each severity category. Surgery under general anaesthesia with appropriate analgesia is a 'moderate' procedure, whereas use of metabolism cages involving severe restriction of movement over a prolonged period (e.g., up to 5 days) is a 'severe' procedure. Most non-human primates on long-term electrophysiology studies will experience a number of surgical procedures during their lifetimes (Pickard *et al.,* 2013), and restraint in a restraint chair for up to 6

[22] http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf

hours per day most days per week (McMillan *et al.,* 2017). The Pickard Committee analysed data extracted from laboratory records on the number of adverse effects and complications experienced by macaques and marmosets used in neuroscience studies predominantly in the UK (Pickard *et al.,* 2013). Infection around implants occurred in 23% of macaques with short-term implants, rising to 39% of macaques with long-term implants. Bone infection occurred in 6% of animals, brain infection in 1%, and cerebral haemorrhage in 1%. The incidence of seizures in studies involving lesions to the central nervous system was 9% of cases. Non-elective euthanasia to end suffering was reported in 26/234 cases, of which 42% were procedure-related. The Pickard data indicates that underestimation of severity can occur (e.g., see Table 9, page 49 for four case studies where the animals failed to cope and premature euthanasia was performed on welfare grounds – identified as clear examples of 'severe' by the UK competent authority but classified by the researcher/s involved as 'moderate').

Prospective severity classification should be made on case-by-case basis and in line with the highest severity that may be experienced by an individual animal. It is important to remember that reuse conditions are based on actual reported severity.

6.6.4.3 Justification for continuing to use NHP in the specific area

A variety of animal species are used in neuroscience. NHPs are generally used where other species such as rodents lack the brain structures of interest, the fine visual and motor abilities under investigation, the use of neurotransmitter systems, the functional connectivity, or the ability to perform complex cognitive and behavioural tasks similar to those used in humans (Jennings, 2016).

There is consensus in the scientific community that one animal model can never fully recapitulate all aspects of human brain diseases. The type of scientific question asked and the methodology used to explore will determine how useful and how predictive the results will be. This implies that a variety of models, animal and non-animal, must be used to address different aspects of the same disease. As an example, mouse transgenic models of Huntington's disease, bearing the same genetic defect as humans, do not exhibit all the major aspects of the disease, whereas non-transgenic NHPs models do (Palfi *et al.,* 2007; Palfi *et al.,* 1996; Pouladi *et al.,* 2013).

The similarity of follow-up techniques used in pre-clinical studies and the appropriate scaling up of the treatment delivered is a very important factor in the predictability of the results for clinical safety and efficacy trials. For gene therapy strategies, assessing adverse events from either off-target infection or deleterious motor consequences such as dyskinesias (abnormal movements) can only be explored in a brain that is as complex as the human brain (e.g., in terms of number of cortical layers) and that presents a similar motor phenotype (which implies a similar connectivity across specific brain regions). For cell therapy, the number of cells needed, the rate of their differentiation (much more rapid in rodents than in NHPs) and the length of the axonal connections are key factors for circuitry reconnection that cannot always be appropriately assessed in small brains before initiating clinical trials (Lindvall, 2016). Perhaps more importantly, the potential adverse effects of these cells (migration/implantation to undesired targets over long distances, overgrowth) can be monitored non-invasively using magnetic resonance imaging (MRI) techniques. The availability of peripheral fluids, in particular cerebrospinal fluid, to follow up adverse events such as rejection and immunogenicity of grafted cells longitudinally using specific immune biomarkers can be challenging in small

animals compared with NHPs and may not predict the clinical scenario (de Lange, 2013).

Imaging techniques like fMRI and PET pharmacology have high translational potential and are increasingly used to identify cell populations involved in disease and these approaches may then be modelled *in silico* or in less complex systems where relevant (Herrmann *et al.,* 2015). The use of NHPs in certain experiments can be justified by the sub-anatomical rearrangement of neuronal populations and their connectivity across brain structures that are not always present or partially present in rodent species like the neocortex, the prefrontal cortex, and visual areas as well as attentional and oculomotor networks (Hutchison and Everling, 2012). Importantly, the connectivity across hemispheres, the presence of homologous structures in both hemispheres (redundancy), or the lateralisation and intra-hemisphere connections are distinctive features in human and NHPs (Passingham, 2009; Stout, 2012; Izpisua Belmonte *et al.,* 2015; Procyk, 2016).

6.6.4.4 Progress made in the last 10 years

There has been extraordinary progress in recent years in the field of Brain-Machine Interfaces (BMIs) that use neuronal action potentials (spikes) recorded by implanted electrode arrays to restore function after paralysis. NHPs have and continue to play an important role, particularly in the development of upper-limb BMIs, since the motor systems controlling the human arm and hand are shared only by other primates (Borton *et al.,* 2013). Key advances made first with NHPs and subsequently translated to paralysed human patients include: the control of computer interfaces in monkeys (Taylor *et al.,* 2002) and humans (Hochberg *et al.,* 2006), the control of robotic prostheses in monkeys (Velliste *et al.,* 2008) and humans (Hochberg *et al.,* 2012), the control of functional electrical stimulation of muscles in monkeys (Ethier *et al.,* 2012) and humans (Bouton e*t al.,* 2016) and artificial sensory stimulation in monkeys (Tabot *et al.,* 2013) and humans (Flesher *et al.,* 2016). In each case, NHP research has driven translation to patients (Bensmaia and Miller, 2014). The next generation of BMIs may act as artificial (neuroprosthetic) connections between disconnected parts of the nervous system (Hampson *et al.,* 2012; Jackson *et al.*, 2006); Hampson *et al.*, 2012; Jackson *et al*., 2006). For example, work is underway to develop methods of reconnecting the brain to the spinal cord (Zimmermann and Jackson, 2014), requiring new spinal cord stimulating technologies which (due to the different neuroanatomy of quadrupeds) must be tested first in NHPs. In addition, improved longevity and stability of BMIs may require new cortical electrodes and signal processing techniques (Hall *et al.,* 2014) which must first be developed and tested in NHPs before they can safely be translated to humans.

Applied studies conducted in animal models, and in particular in NHPs, have also had an important role in the advancement of gene therapy for neurodegenerative diseases (Hocquemiller *et al.,* 2016). Taking Parkinson's disease as an example a gene therapy vector that produces dopamine in the brain has been shown to be safe and efficacious in pre-clinical studies in rodents and NHPs and in the first generation of patients treated in Europe (Jarraya *et al.,* 2009; Palfi *et al.,* 2014).

Finally, immunotherapies and silencing strategies in aggregation diseases like Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis and Frontotemporal Dementia aim at neutralising or eliminating aggregated proteins believed to be pathological and result in neuronal death (Arevalo-Villalobos *et al.,* 2016; Crunkhorn, 2016; Merienne *et al.,* 2015; Nielsen and Nielsen, 2013; Valera and Masliah,

2013). These proteins are naturally present in the brain, have one or more physiological functions that are not completely understood and are present in many different conformations in the brain. While the refinement of these strategies can be tested *in vitro* by making gene silencing more specific or improving the specificity of antibodies targeted against pathological protein epitopes, an assessment of the systemic effects, both beneficial and adverse, of reducing protein load in the whole body has been assessed in NHPs and proven to be safe before testing them clinically (McBride *et al.,* 2011; Grondin *et al.,* 2012; Stiles, Zhang *et al.,* 2012; Grondin *et al.,* 2015; Kordasiewicz *et al.,* 2012; Keiser *et al.,* 2016).

Despite this progress, a 2011 review by an independent panel convened by the major public UK funders of NHP research found little direct evidence of actual medical benefit in the form of changes in clinical practice or new treatments arising from the NHP neuroscience portfolio funded over a ten year period between 1997 and 2006 inclusive (Bateson *et al.,* 2011). There were a number of reasons for this including lack of a genuine link to medicine, the time that had elapsed between the research and the review and a lack of awareness on the part of the researchers about the pathway to medical benefit and how to move discovery to practical application. The panel also noted, however, that the neuroscience was good quality and highly cited. It is important that the justifications offered for individual NHP research projects are soundly based and realistic and any potential health benefits are demonstrable. In addition, there should be effective knowledge transfer and dissemination to ensure maximum benefit.

6.6.4.5 Replacement possibilities

Most behavioural neuroscience studies combine measurements of sensory, cognitive, motor or other psychological functions with some method of monitoring and manipulating the activity of individual neurons, circuits or systems to establish a causal relationship between the brain and behaviour or cognition. Because many of the procedures used to monitor and manipulate brain elements are invasive (such as electrophysiological recording or stimulation of neurons, and production of temporary or permanent lesions via surgical, chemical or physical means), only rarely is it considered ethical for them to be carried out in humans. It has been proposed that many more studies involving recording from single neurons could be made directly in people (Bailey and Taylor, 2016). However, when these studies are conducted, the electrode placement sites are driven exclusively by clinical need (e.g., implanting DBS electrodes) and often the brain tissue under study is compromised by a disease process like epilepsy, Parkinson's disease or traumatic brain injury (Jacobs *et al.,* 2013; Priori *et al.,* 2013; Worrell *et al.,* 2012). Moreover, the recording duration of this type of studies is limited compared to the longitudinal follow-up available in the experimental setting. Hence, their potential for replacing NHP studies is limited. Nonetheless, where opportunities for microelectrode recording exist, with the patient's informed consent, minimal risk and ethics committee approval, they should be utilised (Mamelak, 2014).

The potential to use functional MRI studies in humans to replace some cognitive neuroscience experiments performed in NHPs has been repeatedly proposed. However, there are limitations in the interpretation of the blood oxygenation BOLD signal, that does not reflect neuronal activity at the single cell or small population level, that prevent this technique from explaining the neural correlates of cognitive neuroscience in the healthy and pathological brain (Logothetis *et al.,* 2008; Schridde *et al.*, 2008; Buzsaki *et*

al., 2012; Rigotti *et al.,* 2013; Buckner *et al.,* 2013). fMRI is a powerful non-invasive neuroimaging method to extrapolate fundamental neuronal mechanisms that can be revealed and interpreted by electrophysiology. Both methods are complementary and many laboratories use approaches in NHPs and humans to advance our understanding of the human brain while reducing the numbers of NHPs used in the laboratory (Procyk *et al*., 2016; Passingham, 2009).

Moreover, recent studies have stressed several critical factors concerning statistical approaches used in fMRI studies (Eklund *et al*., 2016), concluding that the widespread use of this technique has to some extent slowed down and mislead current neuroscience research.

Regarding other non-invasive imaging techniques proposed to be able to replace neurophysiological studies in NHP, their limitations have been previously reviewed (Bateson *et al.,* 2011):

"*despite the opportunities offered by imaging techniques, they are a long way from being able to replace completely studies in NHPs and other animals. For example, information on the direction of an anatomical connection (anterograde versus retrograde) is unavailable from MRI-based techniques, and it is impossible to infer fine grained connectivities (at the level of individual cells or groups of cells) using these approaches. Functional mapping techniques such as fMRI are limited in recording a haemodynamic signal, rather than the neuronal activity itself. This makes it impossible, for example, to make inferences about the relative timing of events at a fine temporal scale. Techniques such as magnetoencephalography (MEG) can offer greater insights into neuronal timing and do directly reflect the electrical activity of a region, but the MEG signal reflects synchronised activity across populations of cells, rather than the single cell level information that is available from electrophysiological studies in animals. Non-invasive, reversible interference with localised human brain functions using TMS allows mapping of brain structure-behaviour relationships, providing a powerful alternative to NHP lesion studies. However, TMS can only be reliably targeted to structures on the cortical surface; deep brain structures, or medial cortical areas, are inaccessible to this technique yet are important to study because of their role in many neuropsychiatric disorders*."

Acknowledging the limitations of existing imaging techniques is important to determine the scientific hypotheses that can be tested through them and what experiments can be directly performed in humans (Perry and Singh, 2014; Ruff *et al*., 2008; Schmid *et al*., 2010). Significantly improving the spatial and temporal resolution of non-invasive imaging technologies should be a high priority given the potential, after appropriate validation, to advance the 3Rs in of NHP research (Boto *et al*., 2016). Transcranial focused ultrasound (tFUS) / focused ultrasound neuromodulation (FUN) is an emerging form of non-surgical neuromodulation that confers advantages over existing electro and electromagnetic technologies by providing a superior spatial resolution on the millimeter scale as well as the capability to target sub-cortical structures non-invasively in NHPs and human (Deffieux *et al*., 2013; Lee *et al*., 2016; Leo *et al*., 2016).

The use of microfluidic chambers/brain chips and mixed cell cultures constitutes replacement strategies are important for understanding cellular processes such as cell-to-cell communication, protein trafficking or the role of glial cells, in health and disease. Advances in stem cell biology have significantly contributed to biomedical research in the last 10 years. The use of patient-derived iPS (induced pluripotent stem) cells has

considerably changed the ethical dilemma of using embryonic stem cells to understand differentiation or as a therapeutic option (Santostefano *et al.,* 2015). *In vitro* disease modelling possibilities have expanded thanks to this technology, in particular when monogenetic diseases rather than sporadic diseases are concerned (Avior *et al.,* 2016). These cells can recapitulate some aspects of the disease, just like animal models can, and allow the replacement animals in the study of cellular mechanisms of disease by assessing neuron-to-neuron or neuron-to-glial cell communication in microfluidic chambers for example. Moreover, it allows the replacement of animals in novel drug screening where the molecular target is known and toxicity assays. The challenges remaining are, for example, the use of viruses for the reprogramming of patients cells derived from skin (fibroblasts) or blood (PBMCs), residual somatic memory that can affect differentiation "purity", and the time that neurons and non-neuronal cells can be kept alive after differentiation in order to study their activity (Korecka *et al.,* 2016). The same holds true for "mini-brains" or organoids where neuronal and non-neuronal cells can form networks, but the degree of connectivity and axonal outgrowth remains to be determined. Moreover, given the differentiation states of cells achieved at present, these "brains in a dish" seem more suitable to study neurodevelopment and developmental disorders of the brain than processes involving aged neurons (Bae and Walsh, 2013). The absence of a blood-brain-barrier, a vascular system and an immune system constitute major limitations when investigating complex pathological processes *in vitro*.

6.6.4.6 Reduction and refinement possibilities

Generally within basic neuroscience the number of NHPs used per study is small. For studies where the experimental unit is the neuron, the standard for publication is two animals per condition; if the results obtained from the second monkey confirming the results can be replicated in another animal. Efforts are made to optimise the yield of data per animal and experimental session, for example via the use of multi-electrode arrays, and in some cases to share data and tissues with other researchers. For studies examining the effects of lesions or other interventions within or between groups of animals, sample sizes may be around four per group, depending of factors such as effect size.

Over the last decade, advances in brain imaging technologies and non-invasive electrophysiological methods have furthered efforts to refine and replace NHP investigations in cognitive neuroscience and pharmacology. For example, integration of structural and functional MRI with transient inactivation of targeted brain regions allows studies to be performed without longer-lasting impairments or disability in the animals (Schmid *et al.,* 2010). Transcranial magnetic stimulation (TMS) provides a way of establishing the causal role of particular brain cortical areas in cognitive function, without causing the permanent tissue destruction that has long been the traditional method. Structural MRI is being used to more accurately target recording, stimulation, lesioning and transplantation procedures, yielding better quality data (t'Hart *et al.,* 2006). MRI scans are also being used to produce head restraint devices custom-fitted to the animal's skull, improving integration and stability and reducing the likelihood of infection, bone necrosis and loosening of the device (Mulliken *et al.,* 2015). Functional MRI in NHPs is developing rapidly, offering a powerful tool for directly refining and potentially replacing some electrophysiological studies (Vanduffel and Farivar, 2014, Ortiz-Rios *et al.,* 2015).

Technological developments have also enabled refinement of surgical and other

procedures within the neurosciences. For example, infrared reflection and video-based systems for tracking eye position/movement have replaced the use of surgically implanted scleral eye coils (Kimmel *et al.,* 2012). Screw-mounted headposts and recording chambers machined from single pieces of titanium have in some cases replaced traditional devices using dental acrylic, which create greater defects in the scalp, are less biocompatible and must be maintained assiduously to prevent infection (Adams *et al.,* 2007; www.ciwiki.net). In some studies, non-invasive means of head restraint can replace surgically implanted devices (Hadj-Bouziane *et al.,* 2014, Slater *et al.,* 2016). Use of antimitotic compounds (e.g., 5-flurouracil) have reduced, and in some cases removed, the need for dural scrapes (Spinks *et al.,* 2003). Modern anaesthetics, from which the animals recuperate more rapidly (Bertrand *et al.,* 2017), have enabled more rapid reintroduction of NHPs to the social group, reducing the likelihood of aggression due to disturbance of the group hierarchy (Jennings and Prescott, 2009). Food and fluid control protocols, used to motivate reliable performance on cognitive and behavioural tasks, have been refined to reduce the impact on animal welfare (Prescott *et al.,* 2010; Hage *et al.,* 2014; Gray *et al.,* 2016). Technological advances have resulted in smaller devices for multi-electrode array recordings and the possibility to obtain *in vivo* electrophysiological data wirelessly (Eliades and Wang, 2008; Fernandez-Leon *et al.,* 2015; O'Shea *et al.,* 2017; Yin *et al.,* 2014).

6.6.4.7 Identification of specific research areas

Many large and small animal species have been used to identify anatomical structures and pathways that are relevant to vision, sensation, hearing motor control and cognition. Traditionally, electrophysiological recordings have been performed *in vivo* and brain slices. An alternative that is heavily investigated is the use of optogenetic techniques in which a virus can infect neurons and render them sensitive to light to modulate their activity. Optogenetics has contributed immensely to identifying networks of connected cell populations, functional pathways and circuitry *in vitro* and in the rodent brain both in health and disease in the last decade (Adamantidis *et al.,* 2015; Deisseroth, 2015). An important refinement is that optogenetic experiments in rodents could better inform deep brain stimulation (DBS) treatments to modulate neural activity in pathways that are anatomically relevant to a given disease and combine it with pharmacological modulation for a symptomatic treatment (Luscher and Pollak, 2016). However, there are several technological aspects (light penetration, stable opsin protein expression without cell dysfunction/inflammation, single neuron versus neuronal population modulation, etc.) that currently prevent this technique from being translated to large brains and replacing standard electrophysiology are under investigation in NHPs.

An important factor in the progress of gene therapy, antibodies, PET radiotracers, contrast imaging molecules and drug delivery strategies for neurodegenerative disease is the crossing of the blood-brain-barrier. The efficient passage of these imaging or therapeutic agents into the brain will determine the success of these approaches in the clinic. Focus ultrasound (FUS) uses ultrasound and a systemic injection of microbubbles in the vasculature in combination with anatomical MRI imaging to transiently and focally open the barrier. Given the size of the brain, the potential adverse events (like tissue heating, lesions, and duration of the opening) and the specificity of the cell populations that need to be targeted, animal models, in particular NHPs, might still be required to validate such new approaches (San Sebastian *et al.,* 2013).

More recently, some research groups have been working on direct reprogramming in the rodent brain, using the proliferation of astrocytes in neurodegenerative diseases to reconvert these cells into neurons, thus avoiding an invasive surgical procedure for cell transplantation (Chen *et al.,* 2015). Because the exact role (beneficial *versus* detrimental) of astrocytic/microglial proliferation during inflammatory processes that may be different in rodents and primates, some work to confirm the mechanisms in NHPs may be a requirement before clinical trials in patients.

6.6.5 Other uses

It is not possible to report on all NHP models in this Opinion. Primates are used in behavioural research and in various other areas of biomedical research in addition to the areas focused on in the Opinion. NHP models are used to study inflammatory disorders, aging (Mattison and Vaughan, 2016), developing and evaluating new tracers for neurodegenerative diseases (Golla *et al.,* 2015) and other diseases, developing and evaluating new drugs and therapies against a variety of disorders (Haanstra *et al.,* 2016) and developing gene therapies (Tadin-Strapps *et al.,* 2015). NHPs will mostly likely be required to study the effects and safety issues for stem cell therapy and organoïd transplants.

6.6.5.1 Ophthalmology

6.6.5.1.1 Introduction

Blindness is a debilitation condition affecting more than a million people in Europe alone. The major causes of sight loss are retinal dystrophies, age-related macular degeneration, glaucoma, and diabetic retinopathy. Given the heterogeneity of inherited eye diseases and the impact on public health, efforts have been made at the EU level to create networks for rapid translation of therapies to the clinic (Cuna-Vaz and Zrenner, 2011).

The retina of NHP and man show some unique features (e.g., both NHPs and humans have a *macula lutea*/*fovea*) not found in other mammals (Eichenbaum *et al.,* 2014) and therefore NHPs represent a more relevant model for specific ophthalmology research. Combes and Shah (2016) provide a summary of the scientific advantages of using NHP models such as forward facing eyes, binocular vision and ability to train them to perform complex tasks. In conjunction, they discuss the associated welfare concerns and limitations of using NHPs including the highly invasive nature of some of the techniques and re-use of animals. The paper gives a comprehensive overview of the *in vivo*, *ex vivo*, *in vitro*, computational models and volunteer studies that can be used in vision research. For example, they describe the restricted possibility that species such as fruit fly, zebra fish and amphibia could be used in some circumstances and outline the pros and cons of subcellular, tissue culture, retinal cell cultures, stem cells and brain slices. However, they argue that the potential for relative replacement is more encouraging especially through the use of human stem cells in developing human cell-based models of visual pathways, the availability of suitable *in vitro* models for efficacy testing of new therapies particularly in the retina (Krishnamoorthy *et al.,* 2016; cited in Combes and Shah, 2016). They provide evidence that the rodent visual system displays similarities with the visual pathway of humans and NHPs and that transgenesis has opened up new avenues of research with rodents. Furthermore, they argue that;

'There is an urgent need to improve the methodology for whole organ eye-culturing, and to discover more information about the structure and function of the visual cortex, in order to expedite the development of improved *in vitro* and *in silico* models and simulations as a way of increasing implementation of advanced replacement techniques in vision research' (Combes and Shah, 2016).

6.6.5.1.2 Pertinence of NHP in the specific research area

No currently available *in vitro* or *in silico* model appears to recapitulate the architectural complexity of retinal cells or their complex structural and functional interactions and many animal models have been attempted to mimic ophthalmic diseases like macular degeneration or glaucoma (Chen *et al.,* 2014; Burgoyne, 2015). Many differences in eye anatomy and function exist between rodents and NHP including colour perception, the presence of forward-looking eyes, a binocular processing that gives rise to the perception of depth, the presence of a fovea, saccadic foveation and a divergent specialisation of the spatial visual attention system; the presence of a macula, central part of the retina, that is responsible for visual acuity and colour vision in human and NHPs (Meier and Reinagel, 2013).

6.6.5.1.3 Progress and future research directions

For some of these models, NHP might be indispensable, such as Usher type I syndrome, an inherited disorder causing deafness and blindness. Many transgenic mouse models have been generated since the discovery of the genetic mutations leading to the disease. Although these mouse strains are deaf, none reproduces the retinal degeneration observed in humans. A recent publication argues that unlike mice, NHPs have the five USH1 proteins colocalising at membrane–membrane connection sites between the outer segment of the photoreceptors and surrounding subcellular compartments called the "calyceal processes" (Sahly *et al.,* 2012). A NHP model of this disease is under development by specifically targeting the proteins in these regions through a shRNA silencing strategy delivered by AAV (Institute of Vision, France).

A major axis of research is the restoration of visual perception in patients suffering from degenerative retinopathies who eventually become blind. One example of these conditions is the genetically inherited retinitis pigmentosa (Dalkara and Sahel, 2014). «Artificial retinas» have been shown to functionally replace photoreceptors in patients suffering from retinal degeneration and new devices are currently being developed and tested in NHPs to improve the number of photodiodes, the biomaterials and the optimal morphology of the implant (Stingl *et al.,* 2016; project SIGH AGAIN[23]).

Alternatively, gene therapy and optogenetic light-mediated activation of remaining transfected retinal neurons are ideal for specifically stimulating the cells that have not degenerated and thus, restore electrical stimulation of the optic-brain circuitry (Duebel *et al.,* 2015).

Gene therapy for a different inherited disease, Leber's Congenital Amaurosis, that causes impaired vision from birth and blindness within a decade, has given promising results in patients but seems to be less efficacious than in a dog model and a new viral vector is under investigation in NHPs to improve the clinical results (Bainbridge *et al.,* 2008;

[23]http://www.rpfightingblindness.org.uk/newsevent.php?newseventid=419&tln=newsevents

Kostic *et al.,* 2010). Finally, cell therapy using embryonic stem cells or induced pluripotent stem cells has given promising results in rodent and primate models of retinitis pigmentosa and researchers have now developed cell lines that might be less immunogenic after transplantation by matching the MHC molecules in the cell donor and the recipient (Shirai *et al.,* 2016; Sugita *et al.,* 2016a; Sugita *et al.,* 2016b). Because of the uniqueness of the visual system in primates, it is certain that some of these and novel therapeutic approaches will be tested in NHPs as well as rodents.

6.6.5.2 Transplantation

NHPs have been used as recipients for investigating the fundamental aspects of organ transplantation (Kean *et al.,* 2006). They have been essential for developing various aspects of organ transplantation and prevention of rejection. Insights gained from NHPs in organ transplantation are used in clinical medicine and clinical research. This has reduced the need for NHPs in transplantation research, though NHPs may still be required to evaluate new immunosuppressive therapies and methods to prevent organ rejection (Zeiser and Blazar, 2016).

The success of organ transplantation in patients with organ failure has increased the demand for human cells, tissues and organs in the treatment of human disease and the shortage of organ donors for transplantation is considered to be a major social problem. Only a minority of patients who may benefit from a transplant will be able to receive one and 10 to 20% of patients on the waiting list for organ transplants will die before a donor organ becomes available (UK National Health Service, 2015). In addition, waiting times are growing and are typically 2 to 5 years. Furthermore, as the transplants themselves may also need replacing, this will exacerbate the situation.

In addition to treatment of the terminal failure of organs such as kidney, lung, liver and heart, transplantation is also being seen as a therapy for other diseases such as cystic fibrosis and for patients affected by diabetes and Parkinson disease.

The concept of "xenotransplantation", i.e. organs from animals, was pioneered a century ago, when transplanting human organs was considered ethically controversial. Grafts were, however, quickly rejected, however, because of unknown forces later identified as immune responses.

The unmet and growing demand for human cells, tissues, and organs coupled with recent advances in the science of immunology and molecular biology (e.g., potent immunosuppressive drugs, transgenic techniques) have stimulated a renewed interest in the transplantation of animal cells, tissues, and organs instead of human cells and organs.

The subject of ethics in relation to xenotransplantation has been widely explored (Sykes, 2003). Various animal rights activists are opposed to the idea of xenotransplantation because they maintain that humans do not have right to breed and use other animals for their own needs. While these issues require considerable debate, the accepted opinion is that animals used for research or clinical xenotransplantation must be treated respectfully and humanely, and they must not be used without regulatory approval.

Xenotransplantation does, however, also raise a major public health dilemma. There is a need to balance the potential promise of this technology to alleviate the shortage of human cells, tissues, and organs currently available for transplantation with the risk of potential transmission of infectious agents to the patient, their close contacts, and the

public at large. Experience with human-to-human transplantation has demonstrated the transmissibility of infectious agents from donor to recipient (e.g., Human immunodeficiency virus (HIV), Creutzfeldt-Jacob disease, Hepatitis B virus, and Hepatitis C virus).

There are anatomic and immunologic similarities between NHPs and humans and these similarities can reduce the immunological and other barriers to the survival and adequate functioning of a xenograft in a human host. For these reasons, some investigators have preferred the use of NHPs as potential sources of cells, tissues, and organs for xenotransplantation. However, the structural and functional similarities may also facilitate the transmission of certain infectious agents.

In light of the lack of supply of human organs for transplantation, several alternatives have been investigated and debated. Implantable mechanical devices have been explored in the field of cardiac transplantation. Recently, research has increased in the area of transplanting embryonic cells across species and growing kidneys and endocrine pancreas cells in situ (Mohiuddin, 2014) Organs from pigs have been the focus of much of the research in xenotransplantation, in part because of the public acceptance of using pigs and the physiological similarities between pigs and human and NHPs.

Xenografts have been proposed as appropriate for infants who are physically too small to accommodate organs retrieved from adult or paediatric donors. Additionally, organs from animal sources could be transplanted into patients currently excluded from the human organ transplantation list.

NHPs are not acceptable organ donors for both practical and ethical reasons. In addition to being uncomfortably close to humans on the evolutionary ladder, they also produce few offspring, are slow to mature, and would be difficult to rear under the sterile conditions required to minimise contamination by shared pathogens.

For xenotransplantation research pigs are the optimal candidates for organ donation (Cooper *et al.,* 2016).

6.6.5.3 Barriers against the implementation of alternatives and opportunities to progress

The spirit of EU Directive 2010/63/EU, which lays down the rules on animals used for scientific purposes, is to facilitate and promote the advancement of alternative approaches and to ensure a high level of protection for animals needed for animal testing. To achieve this, it requires Member States to promote the use of 3Rs-principles in scientific research and wherever possible a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure (on animals). This Opinion highlights that in the case of NHPs there are many scientific approaches that could significantly contribute to the replacement, reduction and refinement of NHP studies and tests. However, there are also significant issues that go beyond the scientific rationale, which are preventing the widespread adoption and development of alternatives to NHP laboratory use. This section describes these barriers to the implementation of alternatives and provides some suggestions of the opportunities to overcome them and make progress toward achieving the ethos of the Directive in Europe.

Schiffelers *et al*. (2014) provide a framework for understanding the barriers and drivers associated with the regulatory acceptance and use of 3Rs models in pharmaceutical and

chemical testing more generally, which can be effectively applied to the case of NHP use in science more specifically. They discuss the issues in terms of three levels that have to align in order for an alternative approach to be accepted and implemented;

***micro level*:** consists of the niche in which innovations such as new test methods are developed and tested. Here, drivers and barriers are found relating to the development and validation of 3R models;

meso level: entails a mix of existing rules and regulations, expertise, practices and institutions that strongly influence the acceptance of innovations like 3R models;

macro level: where broader societal features, like the existing material infrastructure, the political culture and coalitions, broad social values, world views, the macro-economy, demography and the natural environment, can be found (Schiffelers *et al.,* 2014, p.42).

In terms of this Opinion, the barriers to NHP alternatives are applicable to animal use more generally but are amplified due to the strong ethical and social concerns surrounding NHP experimentation. In this context, the micro-level barriers are the scientific limitations of alternative methods that have been described in this Opinion as well as the uncertainty of how to translate the findings from such models and build up the necessary knowledge base to refer to. The main meso-level barriers are legislative, in particular, the lack of regulatory harmonisation both within and across sectors and the condition that is often included that that an alternative method must be scientifically valid, justified and accepted (Schiffelers *et al.,* 2014). A second meso level problem is a lack of resources for developing alternatives to NHP models. At the macro level, the potential to replace primates is not just about scientific data but is strongly related/reliant on factors related to scientific practice where dynamics such as competition, the reputation of the researchers and entrenchment and policy create polarisation (Hudson-Shore, 2015; Innovative UK, 2015) and add to the problems at the micro and meso levels. Another major macro-level barrier is the risk aversive nature of society which makes it difficult to move away from familiar methods to new alternative methods where there is less historical data to fall back on.

When considering these barriers and how to overcome them, this Opinion illustrates that there is a great deal of work being conducted at the micro-level with many examples of alternatives to NHP use that have been or are being developed. It also provides the impetus for encouraging innovation and further scientific progress in terms of model and technique development. There have also been a small number of funded European initiatives to further the implementation of 3Rs principles in the field of NHP research including EUPRIMNet[24], ANIM.AL.SEE[25] and PRIMTRAIN[26]. There is an urgent need to conduct systematic reviews and meta-analysis of all areas of primate use. While this will be time consuming in the short-term, in the medium- and long-term, it could result in significantly reducing the number of NHPs used by identifying where they are unsuitable models or where they have contributed very little to current knowledge. This will save resources and animals in conducting further unnecessary studies. It will also provide evidence for much more targeted use of NHPs which could lead to reduction and refinement and will ensure that the scientific justification for their use is much more

[24]http://www.euprim-net.eu/
[25]http://www.inemm.cnr.it/animalsee/index.html
[26]http://www.cost.eu/COST_Actions/ca/CA15131

specific and robust. For advice and guidance on systematic reviews and meta-analyses of animal studies, researchers may wish to consult the NC3Rs/CAMARADES Systematic Review Facility (http://syrf.org.uk/), which includes a free app to help researchers to utilise these methodologies.

At the meso-level, there is still much to be done in terms of ensuring regulations are harmonised and fully implemented to prevent opportunities to replace primates being missed. In particular, it is important that the problems with validation are addressed so that the paradox of *in vitro* models being expected to meet criteria that were never met by most animal tests is resolved. In recent years, there has been some improvement in the resources and funding available for developing alternatives and implementing 3Rs initiatives. However, this is still small in comparison to the figures involved in animal based-research. In addition, Research Councils and Funding Bodies should conduct regular reviews of the outcomes of their NHP projects to ensure that the work has resulted in a significant enough outcome to justify the use of NHPs and that funding is being effectively distributed. While not perfect, the Bateson Report (2011) provides an example of the kind of review, which can be conducted.

The macro-level barriers are perhaps the most difficult to address as they require changes in attitude both scientific and societal and in scientific practice. Schiffelers *et al.* (2014), Taylor (2014) acknowledge these difficulties, but they provide a core set of tools to enhance the process of aligning the levels and moving the process forward which is very applicable to NHP use. They advocate the 4Cs;

Commitment: This includes an international commitment to 3Rs models for scientific and ethical reasons as well as the practical commitment of allocation of resources for the development, validation and implementation of 3Rs models.

Communication: Finally, NHP researchers should have access to training opportunities to help them communicate the aims and impacts of their research to the general public. The Concordat on The Concordat on Openness initiative in the UK is a welcome development, which is being replicated in other EU countries (e.g. Tierversuche Verstehen Initiative in Germany and GIRCOR in France). Organisations signing the Concordat agree to be more open and transparent about their animal research, to provide accurate descriptions of the benefits, harms and limitations, be realistic about the potential outputs of such research, and be open about its impact on animal welfare and the ethical considerations involved. The Bateson Committee (Bateson *et al.,* 2011) also commented on public engagement, cautioning against overstating and generalising the medical benefit of NHP research given that much of it is funded primarily for its scientific value.

Cooperation: Cooperation or collaboration is arguably the most important means by which to progress 3Rs initiatives in NHP use. Schiffelers *et al.* (2014) summarise it as the process where two or more parties interact to create shared understanding. It is not only about exchanging information but also about education and the mutual use of information. It is important at all stages in the 3Rs process. This opinion includes several examples where such cooperation has been very effective such as the NC3Rs working groups on NHP use in mAb development and vomiting and nausea, and the EUPRIM-Net initiative, which facilitated the implementation of the 3Rs among NHP researchers from different lines of research. Cooperation needs to be inter and intra-disciplinary and needs to cut across different stakeholder sectors such as regulators, industry and animal

protection groups to drive innovation and change.

Coordination: This is needed to guide alternatives to NHP models through the chain from models development to the ultimate implementation in regulatory guidelines and/or everyday scientific practice. This will involve the cooperation detailed above on an international as well as national scale and the creation of clear roadmaps to follow. Communication will be key to successfully and efficiently completing each part of the chain.

7 RECOMMENDATIONS FOR FURTHER WORK

Some of the recommendations given below are already requirements of EU Directive 2010/63, however they are incorporated into the committees series of recommendations to emphasise their particular importance in relation to the use of non-human primates, and to encourage their full and rapid implementation in all member states.

7.1 Advancing 3Rs

R01 Decisions about the need for NHPs in research projects or regulatory testing should be made case-by-case based on strong scientific rationale and the availability of alternative approaches. For instance, it may be possible that data from one species is sufficient for progression of a potential new drug into human clinical trials. Although international regulations for drug safety testing specify that non-rodent species should be used and that one of these species can be NHPs, a significant change is consideration of the need to use NHPs on a case-by-case basis. The choice of species for a research project should, in each case, be based on scientific data on the most appropriate model. There should also be harm-benefit assessment taking into account the importance of the biological or medical question and the anticipated benefits, the quality of experimental design and likelihood of successfully answering the question, and ethical considerations, including the number of animals involved and the harm caused to them. The EC and others have published guidance on how to perform robust harm-benefit assessments (European Commission, 2013; Home Office, 2015; Brønstad *et al*., 2016; Laber *et al*., 2016).

R02 In contrast to current areas of research with great potential to replace current NHP use such as in safety testing of pharmaceuticals, other areas of research, partly new, may require increased NHP use in the future, e.g., emerging infectious diseases. A solid harm-benefit assessment is needed here, too. The majority of NHP use in the EU is for safety assessment studies, performed to meet regulatory requirements. NHPs should only be used when there are no alternatives and it is scientifically demonstrated that none of the other non-rodent species commonly used in safety testing is appropriate for the purpose of the study. In addition NHP use can be avoided when *in vitro* preliminary studies demonstrate NHP are not a suitable animal model. It is indeed important to consider the limitations of the NHP when choosing which species to use in drug safety test: the use of an appropriate species or combination of species/models is essential to obtaining the most reliable and translatable information.

R03 Progress has been made in identifying opportunities to avoid NHP use where they are not a relevant species or alternative species can be used, and in identifying efficient study designs using fewer animals where NHP use is scientifically justified. This progress has largely been led by the NC3Rs working in collaboration with industry companies and regulatory bodies internationally, acting as a honest broker

for pre-competitive data sharing and providing an open forum for dialogue between the parties. The evidence base and recommendations are being put into practice, but there is scope for wider uptake across the industry to further advance the 3Rs.

R04 Research funders and ethics committees should ensure that research is conducted on NHP only where there is no suitable alternative approach, and where there is a high likelihood of producing scientific, medical or social benefit. This may require improvements to existing peer review processes to make them more robust. It is recommended that an international working group develops a clear listing of the elements that should be scrutinised by project evaluators for their authorisation and/or funding including experimental design and systematic inclusion of a publication plan (including for negative results) for non-regulatory projects. Researchers should report their NHP studies in compliance with the ARRIVE Guidelines in order to maximise the information published and avoid unnecessary studies.

R05 With regard to transgenic techniques (e.g., CRISPR) in NHPs, the SCHEER recommends that the European Commission form a working group to assess the scientific and ethical implications of such research to determine if it should be allowed in the EU and, if so, within what constraints.

R06 Conduct systematic reviews in all areas of NHP research, where possible, to conclude on its value, translational relevance and necessity in the context of alternative approaches. Cross-company and cross-sector data sharing would be a path forward for this type of retrospective assessment of NHP studies.

R07 Where NHP research is necessary and justified, it should be performed to genuinely high standards of experimental design and technical practice, ethics and animal welfare. Experiments should be performed by appropriately trained and skilled staff with the necessary knowledge, resources and infrastructure to fully implement the 3Rs, as required by legislation and expected by general public. To this end, we recommend that consideration be given to focusing NHP research in centres of excellence and improving existing networks for information sharing.

R08 Researchers, ethics committee members, animal welfare committee members and animal care staff must ensure that they keep abreast of the latest techniques that enable reduction in animal numbers and the refinement of methods and techniques to reduce suffering, and put this evidence base into practice. Ideally, this is a continuous process of improvement and CPD. Institutional constraints on implementation of the 3Rs should be recognised and addressed. Training should be included in non-animal methods to improve skills in multidisciplinary science and technology can help drive the development of non-animal technologies by training researchers in new skills for continuing their research in their field, without animal use.

R09 CPD should be seen as an essential part of the training process for research workers using NHPs. Researchers, veterinarians and animal technicians should make full use of the CPD opportunities available, and this should be supported by their research organisation. CPD should form part of training records and assessment of competency.

R10 Available training opportunities are fragmented and not always aligned with best practice. Consideration should be given to development of an accredited, harmonised training course for those involved in NHP research to provide a solid foundation in NHP behaviour and best practice in their care and use.

R11 Funding should also be provided for exchange visits and practical workshops for those directly involved in the care and use of NHPs to facilitate sharing of best practice.

R12 Whilst there has been progress with refinement of neuroscience studies involving NHPs, funders and researchers should focus on significantly refining devices and methods as well as improving the spatial and temporal of existing and new non-invasive imaging technologies to refine, validate and ultimately replace the use of highly invasive techniques in NHP. Experiments should make full use of modern imaging, biotelemetry, virtual models and other technologies, and sharing of data and resources (animals, tissues and equipment) between researchers and institutions, to reduce and refine NHP use.

R13 There needs to be improved means of assessing pain and distress in NHPs, including the psychological impact of their use in research. Scientific knowledge about the welfare impact of husbandry and procedures, even after refinement measures have been applied, needs to be factored into harm-benefit assessments.

R14 Reduction in NHP use would be assisted by greater use of more efficient experimental designs (e.g., factorial designs). Careful consideration should also be given to sample size calculations for NHP studies. The NC3Rs Experimental Design Assistant (https://eda.nc3rs.org.uk/) is one source of tailored advice for those lacking institutional access to expert statistical support.

R15 Breeding facilities should contribute to improvements in both animal welfare and quality of science by ensuring that animals are well habituated to humans and better able to cope with scientific and husbandry procedures.

R16 Literature searches, mainstream scientific and specialist conferences and resources from institutions like the NC3Rs are all sources of information and advice on opportunities to apply the 3Rs and the benefits of this. Researchers and associated animal care staff should ensure that they continually review and adapt their working practices accordingly.

R17 For the sake of transparency and monitoring progress in the application of the 3Rs, it would be desirable that all Member States report on the level of severity of experimental procedures, origin of animals, generation and first time use.

7.2 How to overcome barriers?

R18 To overcome the wider social, non-technological barriers to the implementation of alternatives it will be necessary to stimulate changes in both scientific and societal attitudes and in scientific practice by improving and expanding the 4Cs (Commitment, Communication, Cooperation and Coordination). This will influence the risk aversive nature of society that makes it difficult to move away from familiar methods to new alternative methods, where there is less historical data to fall back on. The 4Cs will also help to reduce problems related to competition, the reputation of researchers and entrenchment and policy create problems.

R19 When communicating about NHP use with the public, the scientific community should provide an accurate description of the benefits, harms to animals and limitations of such research, and be realistic about the potential outputs and impacts. For safety testing, regulatory requirements and scientific consideration, it is possible to use NHPs, with proof that NHPs are most representative of humans, regarding pharmacodynamics and pharmacokinetics.

R20 Researchers should report their experimental methods and results comprehensively,

accurately and transparently. They should transparently evaluate and report on the progress in the development of alternative methods and their validation, but also on scientific limitations of alternative methods as well as the uncertainty of how to translate the findings from such models and build up the necessary knowledge base to refer to.

R21 It is also necessary to reduce the timescale and bureaucracy associated with the process of formal validation and to overcome the lack of regulatory harmonisation both within and across sectors.

R22 The EC should instigate strategic research funding initiatives to support the scientific and technological development required to achieve NHP replacement, or at least considerable progress towards it. This would also help the scientific community meet the policy objectives of the Commission.

R23 To progress towards complete replacement of NHPs in safety testing, it will be necessary to gain advance molecular biology techniques, including, for example, a better understanding of signalling pathways, mode-of-action information, modelling and bioinformatics. By integrating these data and the results of *in vitro* testing, — omics technologies applied to *in vitro* systems and physiologically-based pharmacokinetic modelling, non-animal models will together more closely represent what happens in a human body.

8 CONSIDERATION OF RESPONSES RECEIVED IN PUBLIC CONSULTATION

A public consultation on this Opinion was opened on the website of the Scientific Committees from 10 February to 26 March 2017. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

The public consultation involved 190 contributors from Academia, researchers, Member States, Non-Governmental Organisations and industry, providing a total of 318 comments, each of them addressing several issues. Each submission was carefully considered by the SCHEER and the Scientific Opinion and biblography was revised accordingly. In addition, a public hearing took place on 14 March 2017[27]. 19 organisations participated in the public hearing.

The comments from the public consultation and SCHEER responses are available at:

https://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scheer_consultation_03_en

9 ABBREVIATIONS AND GLOSSARY OF TERMS

3Rs	Replacement, Reduction, Refinement
4Cs	Commitment, Communication, Cooperation and Coordination
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ABPI	Association of British Pharmaceutical Industry
BMIs	Brain-Machine Interfaces
CHMP	European Committee for Medicinal Products for Human Use
CNS	Central Nervous System
CPD	Continuing Professional Development
DG ENV	Directorate-General for Environment in the European Commission
DBS	Deep Brain Stimulation
DILI	Drug-Induced Liver Injury
EDA	Experimental Design Assistant
EFSA	European Food Safety Authority
EMA	European Medicines Agency

[27]https://ec.europa.eu/health/scientific_committees/events/ev_20170314_en

EPAR	European Public Assessment Report
EC	European Commission
EU	European Union
FDA	US Food and Drug Administration
FRAME	UK Fund for the Replacement of Animals in Medical Experiments
FUS	Focus ultrasound
IACUCs	Institutional Animal Care and Use Committees
IADRs	Idiosyncratic Adverse Drug Reactions
ICH	International Council for Harmonisation
iPSCs	Induced Pluripotent Stem Cells
mAbs	Monoclonal Antibodies
NC3Rs	UK National Centre for the Replacement, Refinement and Reduction of Animals in Research
NHPs	Non-human primates
PFC	Prefrontal Cortex
PRIMTRAIN	European Network Behavioural Management and Training of Laboratory non-human Primates and Large Laboratory Animals
SC	Scientific Committee
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHEER	Scientific Committee on Health, Environmental and Emerging Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCCS	Scientific Committee on Consumer Safety
TB	Tuberculosis
TMS	Transcranial magnetic stimulation
WMA	World Medical Association
WG	Working Group

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ANNEXES

Annex I- Definitions and examples of replacement, reduction and refinement[1]

'R'	Definition	Examples
Replacement	Methods that avoid or replace the use of animals in areas where they would have otherwise been used. In some cases, relative replacement (i.e. replacing the use of live 'protected' vertebrates with vertebrate cells or tissues, early life-stages or non-vertebrates) has been implemented as a first step to absolute replacement.	Human volunteers, tissues and cells; mathematical and computer models; established animal cell lines, or cells and tissues taken from animals killed solely for this purpose (i.e. not having been subject to a regulated procedure); non-protected immature forms[2] of vertebrates; invertebrates, such as *Drosophila* and nematode worms
Reduction	Methods that minimize the number of animals used per experiment or test, either by enabling researchers to obtain comparable levels of information (of a given amount and precision) from fewer animals, or to obtain more information from the same number of animals (thereby avoiding further animal use).	Improved experimental design and statistical analysis; sharing of data and resources (e.g., animals and equipment) between research groups and organizations; use of technologies, such as imaging, that enable longitudinal studies in the same animals.
Refinement	Methods that minimize any pain, suffering, distress or lasting harm that may be experienced by the animals, and improve animal welfare. Refinement applies to all aspects of animal use, from the housing and husbandry used to the scientific procedures performed upon them.	Use of appropriate anaesthetics and analgesics regimens; avoiding stress by training animals to cooperate with procedures such as blood sampling; providing animals with appropriate housing and environmental enrichment which allows the expression of species-specific behaviours.

[1] Reproduced from Graham ML, Prescott MJ (2015) The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease. European Journal of Pharmacology 759, 19-29.

[2] In the European Union, non-protected immature forms are embryonic and fetal mammals, birds and reptiles up to the last third of their gestation or incubation period, larval forms of amphibians and fish until they can feed independently, and cephalopods until the point at which they hatch.

Annex II - Publically available information concerning the publication of statistical data under Article 54(2) of Directive 2010/63/EU

AT	http://wissenschaft.bmwfw.gv.at/fileadmin/user_upload/forschung/recht/tierversuche/Tierversuchsstatistik_2014.pdf
BE	Walloon: http://environnement.wallonie.be/bea/ANIMAUX-EXPERIENCES-WALLONIE-2014.pdf Brussels: http://document.environnement.brussels/opac_css/elecfile/IF_Statistiques_Bien-etre_animal_NL Flanders: http://www.lne.be/themas/dierenwelzijn/proefdieren-in-vlaanderen-statistieken-2014
BG	http://babh.government.bg/userfiles/files/ZHOJKF/Used%20lab.%20animals%20in%20BG%20for%202014.pdf
CY	http://www.moa.gov.cy/moa/vs/vs.nsf/All/6C4016CA75D69447C2257F7D0043ADEF/$file/annual%20report_2014.pdf
CZ	http://eagri.cz/public/web/mze/ochrana-zvirat/aktualni-temata/pokusna-zvirata/prehled-zvirat-pouzitych-k-pokusum/tabulky/
DE	http://www.bmel.de/DE/Tier/Tierschutz/_texte/TierschutzTierforschung.html?docId=7027766 http://www.bmel.de/SharedDocs/Downloads/Tier/Tierschutz/2014-TierversuchszahlenGesamt.pdf;jsessionid=64CCE2048E4885C805259F03AF3B9FBD.2_cid376?__blob=publicationFile
DK	www.dyreforsoegstilsynet.dk
EE	http://www.agri.ee/et/loomkatse-korraldamine
EL	http://www.minagric.gr/images/stories/docs/agrotis/zoika_ypoproionta/plhrofories_diadikasias_zoon2014.pdf
ES	http://www.magrama.gob.es/gl/ganaderia/temas/produccion-y-mercados-ganaderos/bienestanimal/en-la-investigacion/Informes_y_publicaciones.aspx
FI	http://www.laaninhallitus.fi/lh/etela/hankkeet/ellapro/home.nsf/pages/BFD5CAFA94D8E7C7C225728A00475B11?opendocument http://www.laaninhallitus.fi/lh/etela/hankkeet/ellapro/home.nsf/pages/5EEDF1897D17F5FFC2257EAD00484621 http://www.laaninhallitus.fi/lh/etela/hankkeet/ellapro/home.nsf/files/Käyttötilasto%202014%20-%20lajit%20vakavuusluokat%20käyttötarkoitukset%20muu%20käyttö/$file/Käyttötilasto%202014%20-%20lajit%20vakavuusluokat%20käyttötarkoitukset%20muu%20käyttö.pdf
FR	http://www.enseignementsup-recherche.gouv.fr/cid70613/enquete-statistique-sur-l-utilisation-des-animaux-a-des-fins-scientifiques.html
HR	http://www.veterinarstvo.hr/default.aspx?id=64
HU	https://www.nebih.gov.hu/szakteruletek/szakteruletek/aai/kozerdeku_aai/kotelezoen_nyilvantartott/allatkiserlet/statisztikai_adatok
IE	http://www.hpra.ie/docs/default-source/publications-forms/newsletters/statistical-report-sap-2014.pdf?sfvrsn=10
IT	http://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2016-08-24&atto.codiceRedazionale=16A06256&elenco30giorni=false
LT	http://vmvt.lt/node/607 http://vmvt.lt/sites/default/files/2014_m_bandomuju_gyvunu_statistine_ataskaita.pdf
LV	http://www.pvd.gov.lv/lat/kreis_izvlne/veterinr_uzraudzba/majas_un_izmeginajumu_dzivniek/izmeginajumu_projektu_netehnis/publiskie_parskati
LU	http://www.ma.public.lu/ministere/rapport/index.html
MT	No animals were used in Malta in 2014
NL	https://www.rijksoverheid.nl/documenten/jaarverslagen/2016/03/01/zo-doende-2014

PL	http://www.bip.nauka.gov.pl/sprawozdania_zwierzeta/
PT	http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?generico=1149097&cboui=1149097
RO	http://www.ansvsa.ro/documente/admin/Statistica%202014_49571ro.pdf
SE	Not yet published.
SI	http://www.uvhvvr.gov.si/si/delovna_podrocja/dobrobit_zivali/zascita_zivali_v_poskusih/
SK	http://www.svps.sk/dokumenty/zvierata/VPHU_1_2015.pdf
UK	UK: https://www.gov.uk/government/statistics/statistics-of-scientific-procedures-on-living-animals-great-britain-2014 Northern Ireland: https://www.dhsspsni.gov.uk/sites/default/files/publications/dhssps/asp-statistics-of-scientific-procedures-on-living-animals-ni-2014.pdf